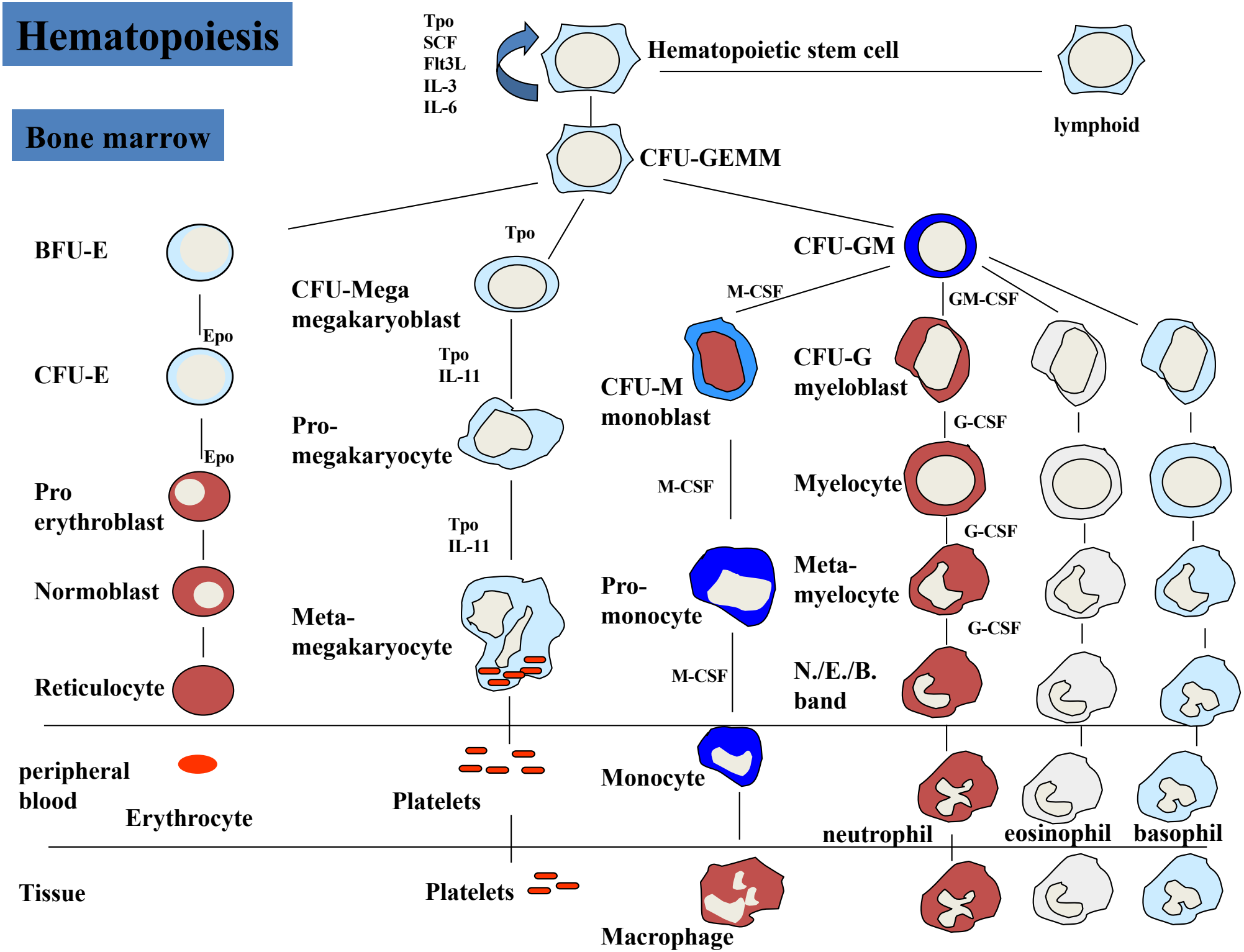
Medicina personalizata in hematologie: perspective

Stefan N. Constantinescu

Hematopoiesis

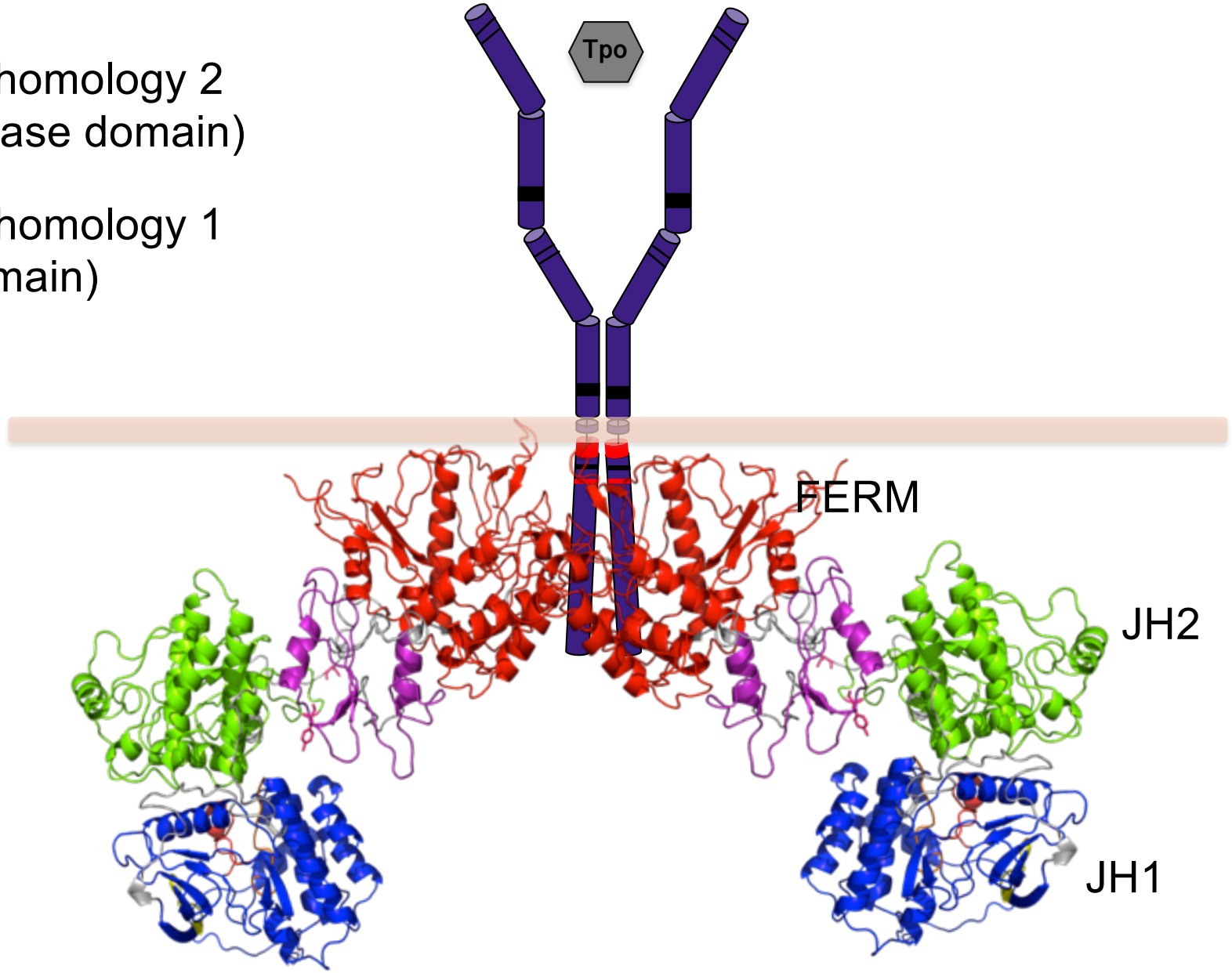
Bone marrow

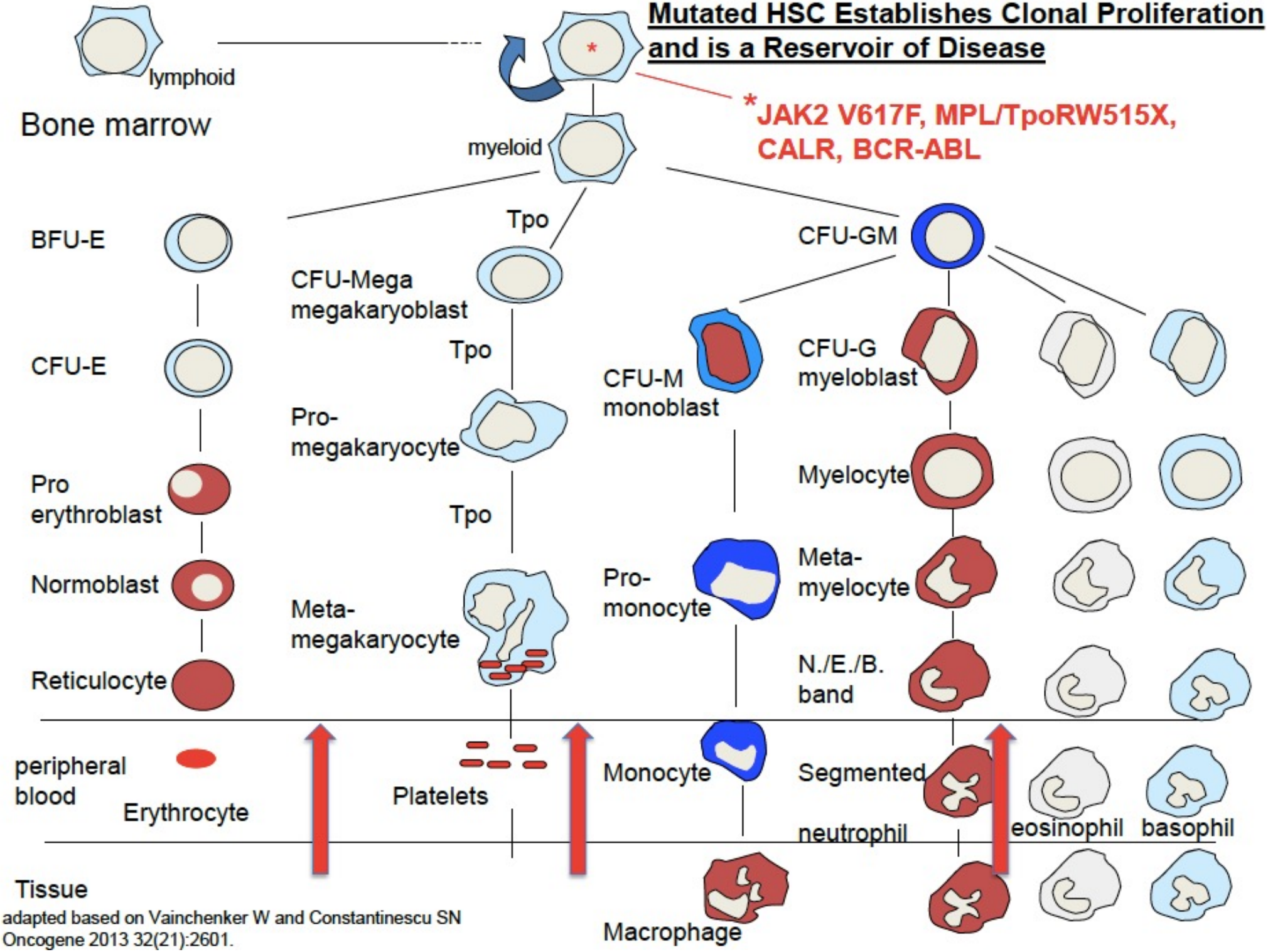


Cytokine Receptor and JAKs Form Functional Units

JH2= JAK homology 2
(pseudokinase domain)

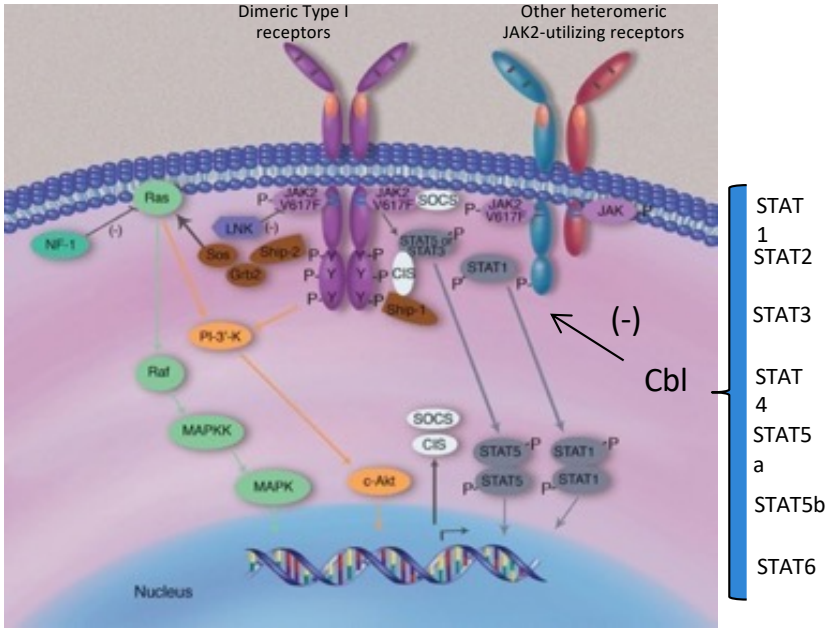
JH1= JAK homology 1
(kinase domain)




Mutated HSC Establishes Clonal Proliferation and is a Reservoir of Disease
*JAK2 V617F, MPL/TpoRW515X, CALR, BCR-ABL
lymphoid
Bone marrow
myeloid
Tpo
BFU-E
CFU-Mega megakaryoblast
CFU-GM
Tpo
CFU-E
CFU-M monoblast
CFU-G myeloblast
Pro-megakaryocyte
Tpo
Pro erythroblast
Myelocyte
Tpo
Normoblast
Meta-myelocyte
Pro-monocyte
Meta-megakaryocyte
Reticulocyte
N./E./B. band
peripheral blood
Erythrocyte
Platelets
Monocyte
Segmented neutrophil
eosinophil
basophil
Tissue
Macrophage
adapted based on Vainchenker W and Constantinescu SN Oncogene 2013 32(21):2601.

Mutations in MPNs

Phenotypic Drivers



Gene	PV[1] (%)	ET[1] (%)	MF[1] (%)	MF[2] (%)
TET2	16	7	9	9.7
IDH1/2	3	0	0	2.6
DNMT3A	3	9	3	5.7
EZH2	2	1	9	5.1
ASXL1	3	1	18	21.7

Clonal dominance and progression

Polycythemia Vera

97%	***JAK2* V617F**
3%	***JAK2* ex 12**

Essential Thrombocythemia

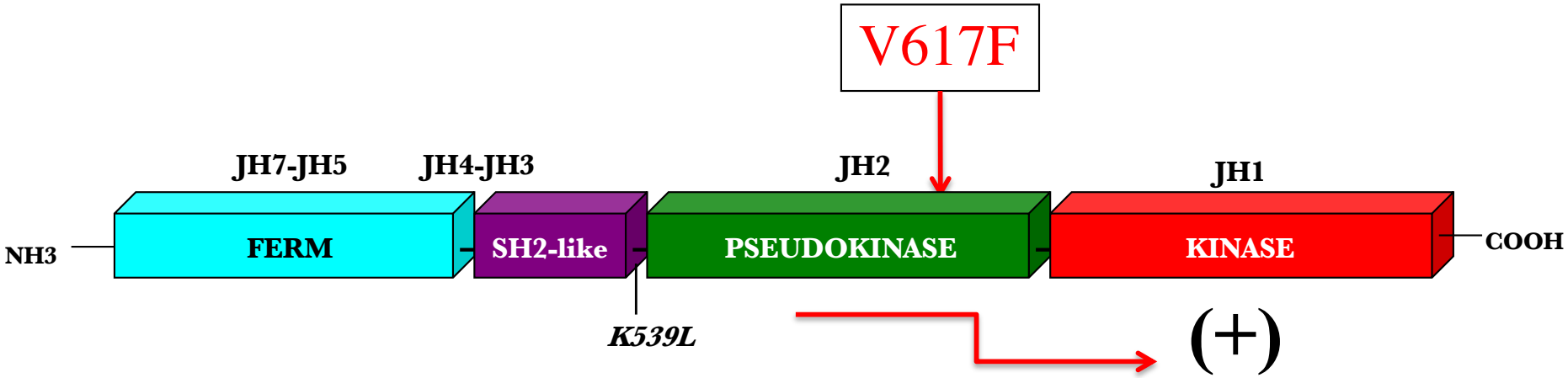
64%	***JAK2* V617F**
4.3%	***MPL* W515X**
<5%	***LNK (SH2B3)***
15.5%	***CALR***
16.1%	**Unknown**

Myelofibrosis (PMF, sMF)

55-64.7%	***JAK2* V617F**
4%	***MPL* W515X**
≤6%	***CBL***
<5%	***LNK (SH2B3)***
22.7-27%	***CALR***
8.6%	**Unknown**

1. Lundberg P et al. Blood. 2014;123:2220-2228.
2. Vannucchi A et al. Leukemia. 2013;27:1861-9.

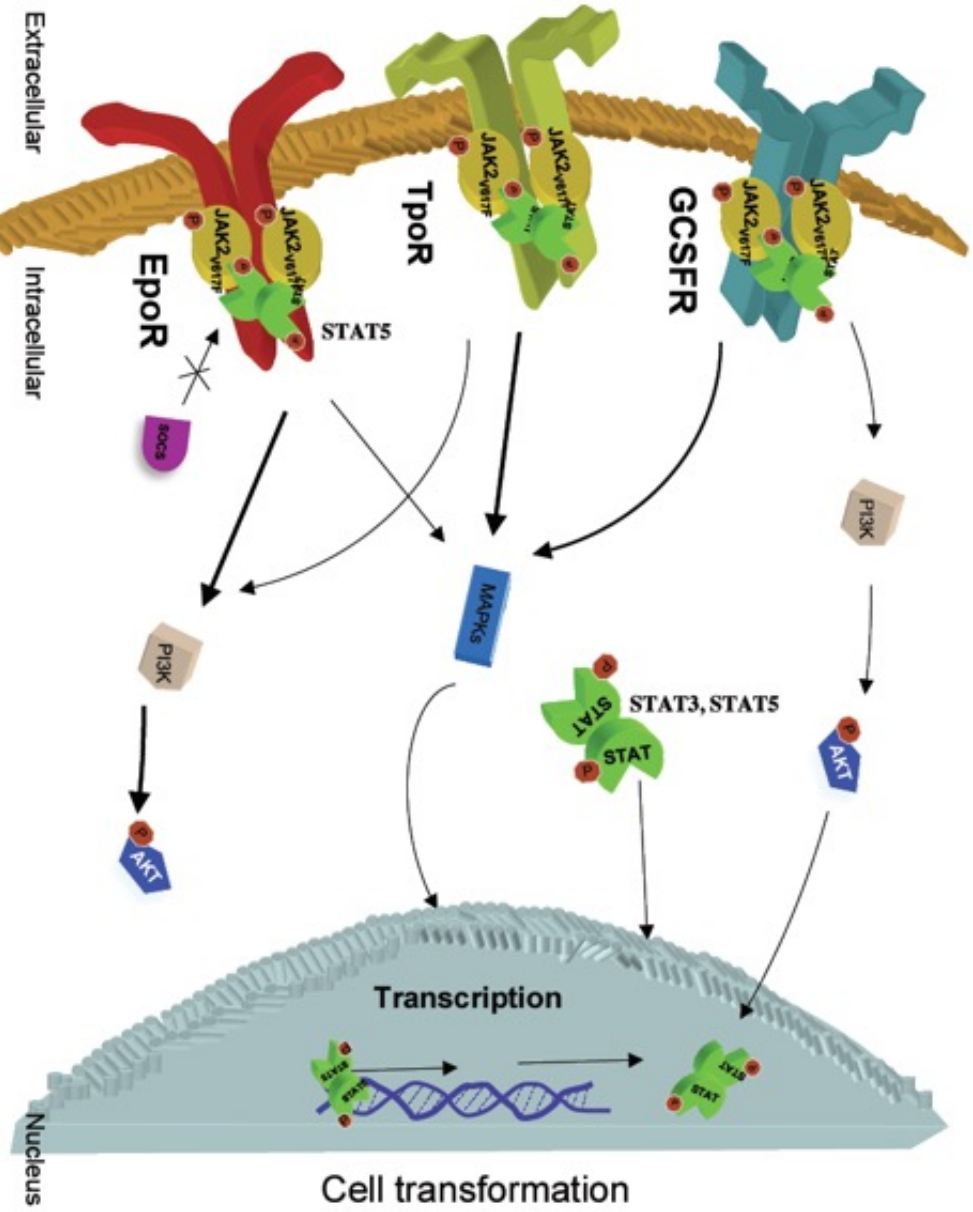
Structure of Janus Kinases (Just Another Kinase)



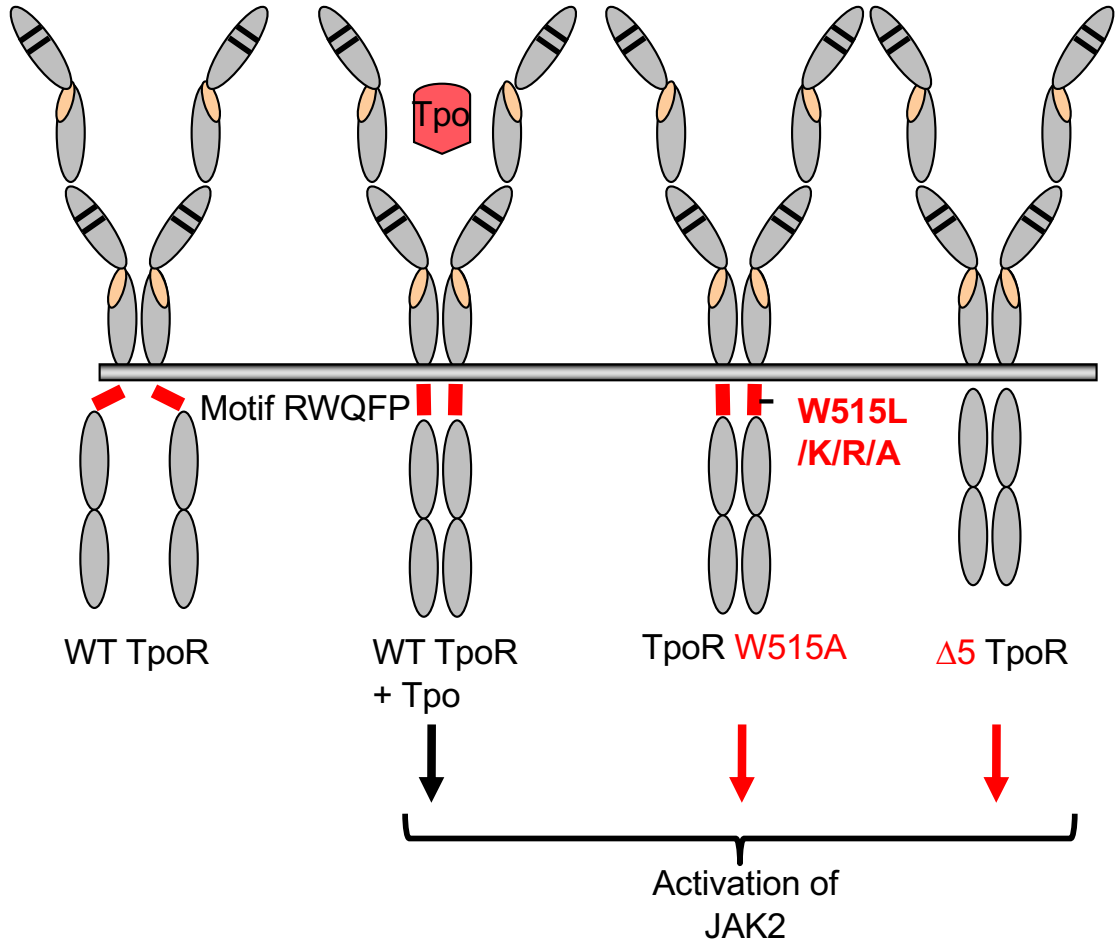
JH1: Kinase domain
JH2: Pseudokinase domain
JH7-4: FERM domain
JH4-3: SH2 domain

James C et al., Nature. 2005 Apr;434(7037):1144-8, Kralovics R et al., N Engl J Med. 2005 Apr;352(17):1779-90, Baxter EJ et al., Lancet. 2005 Mar;365(9464):1054-61., Levine R et al., Cancer Cell. 2005 Apr;7(4):387-97

JAK2 V617F Binds to and Activates EpoR, TpoR and G-CSFR



Myeloproliferative Neoplasms Induced by W515 Mutations in TpoR



Staerk J et al., *Blood* 2006, 107, 1864

Pikman Y et al., *Plos Med* 2006, 3, e270

Pardanani AD et al., *Blood* 2006, 108, 3472

Beer PA et al., *Blood* 2008, 112, 141

Pecquet C et al., *Blood* 2010, 115, 1037

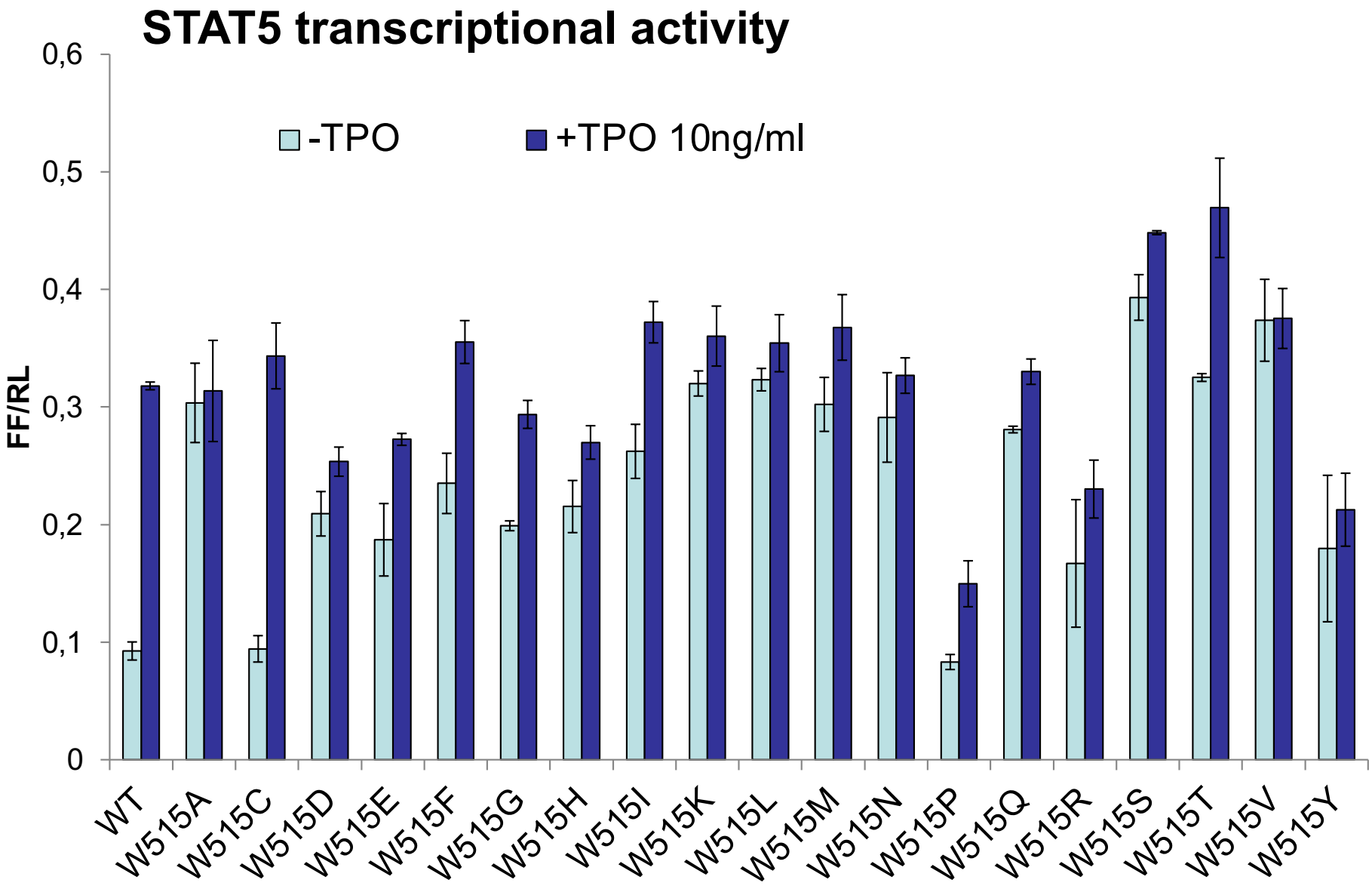
HEMATOPOIESIS

An amphipathic motif at the transmembrane-cytoplasmic junction prevents autonomous activation of the thrombopoietin receptor

Judith Staerk, Catherine Lacout, Takeshi Sato, Steven O. Smith, William Vainchenker, and Stefan N. Constantinescu

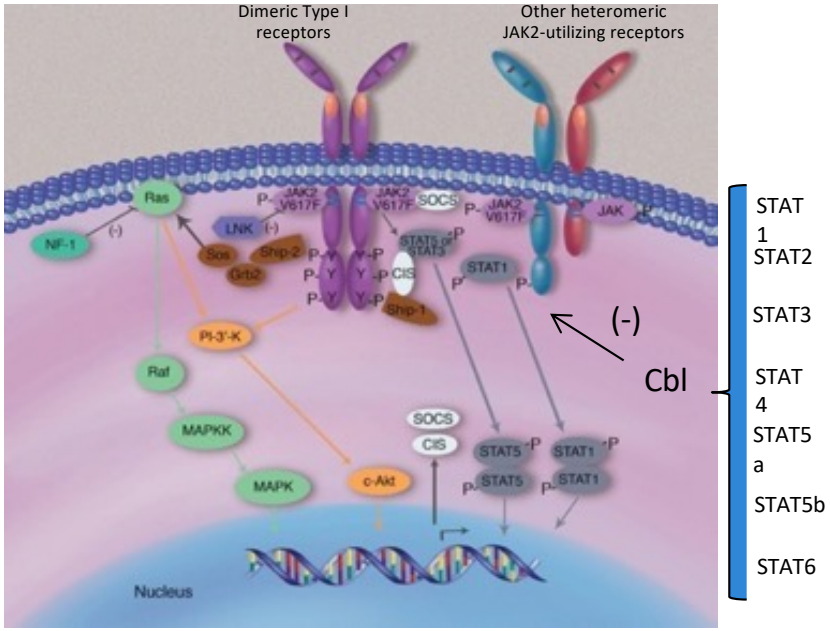
(Blood. 2006;107:1864-1871)

Tryptophan is “Absolutely” Required at Juxtamembrane Position 515 in Order to Maintain the Unliganded TpoR Inactive



Mutations in MPNs

Phenotypic Drivers



Gene	PV[1] (%)	ET[1] (%)	MF[1] (%)	MF[2] (%)
TET2	16	7	9	9.7
IDH1/2	3	0	0	2.6
DNMT3A	3	9	3	5.7
EZH2	2	1	9	5.1
ASXL1	3	1	18	21.7

Clonal dominance and progression

Polycythemia Vera

97%	***JAK2* V617F**
3%	***JAK2* ex 12**

Essential Thrombocythemia

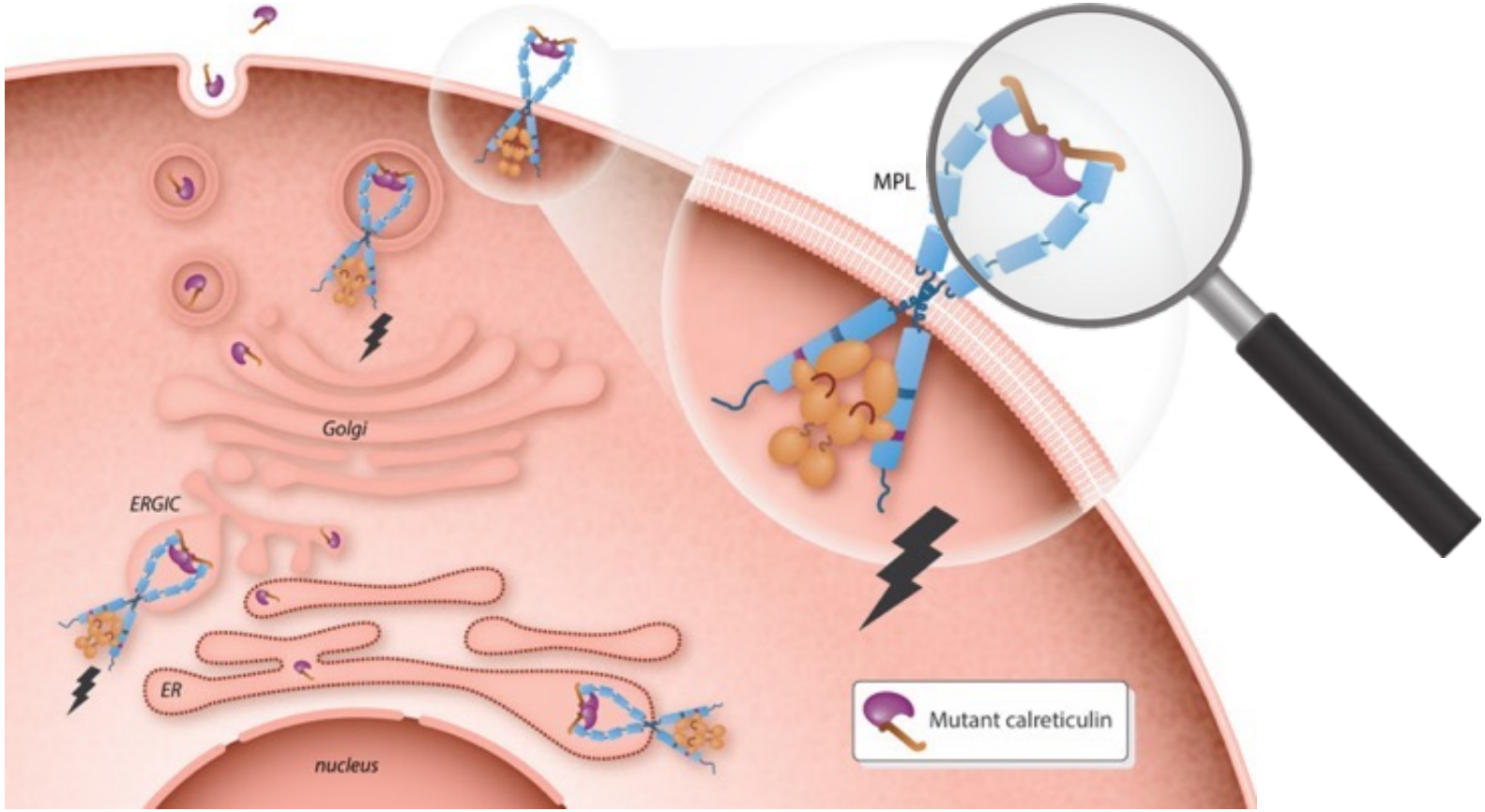
64%	***JAK2* V617F**
4.3%	***MPL* W515X**
<5%	***LNK (SH2B3)***
15.5%	***CALR***
16.1%	**Unknown**

Myelofibrosis (PMF, sMF)

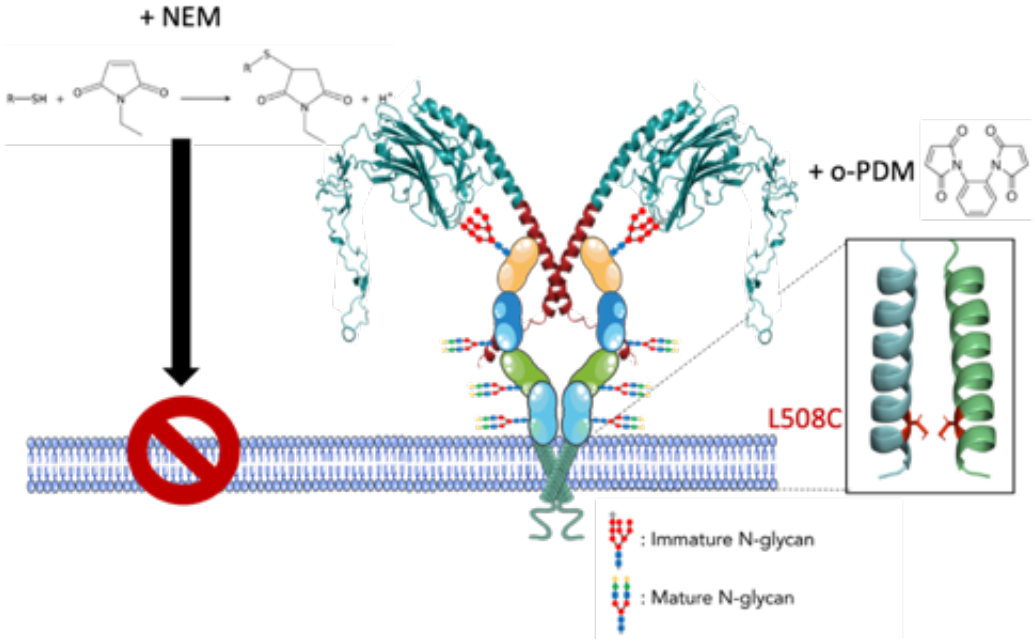
55-64.7%	***JAK2* V617F**
4%	***MPL* W515X**
≤6%	***CBL***
<5%	***LNK (SH2B3)***
22.7-27%	***CALR***
8.6%	**Unknown**

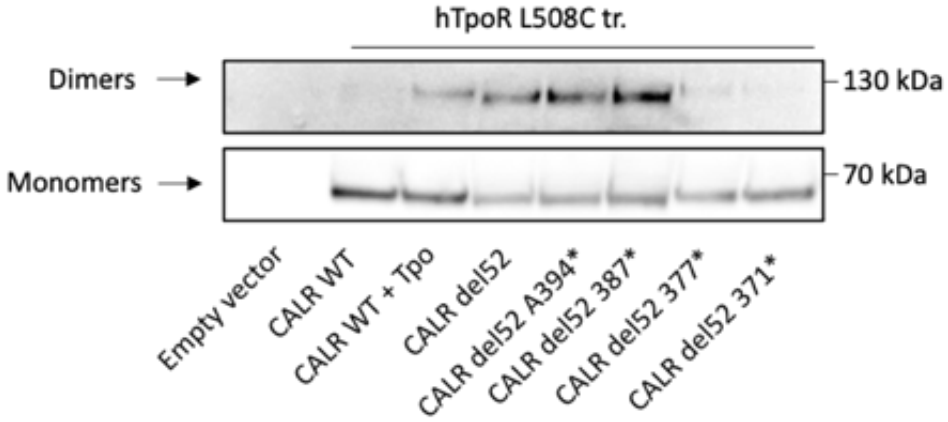
1. Lundberg P et al. Blood. 2014;123:2220-2228.
2. Vannucchi A et al. Leukemia. 2013;27:1861-9.

CALR del52 binding and activation of human TpoR



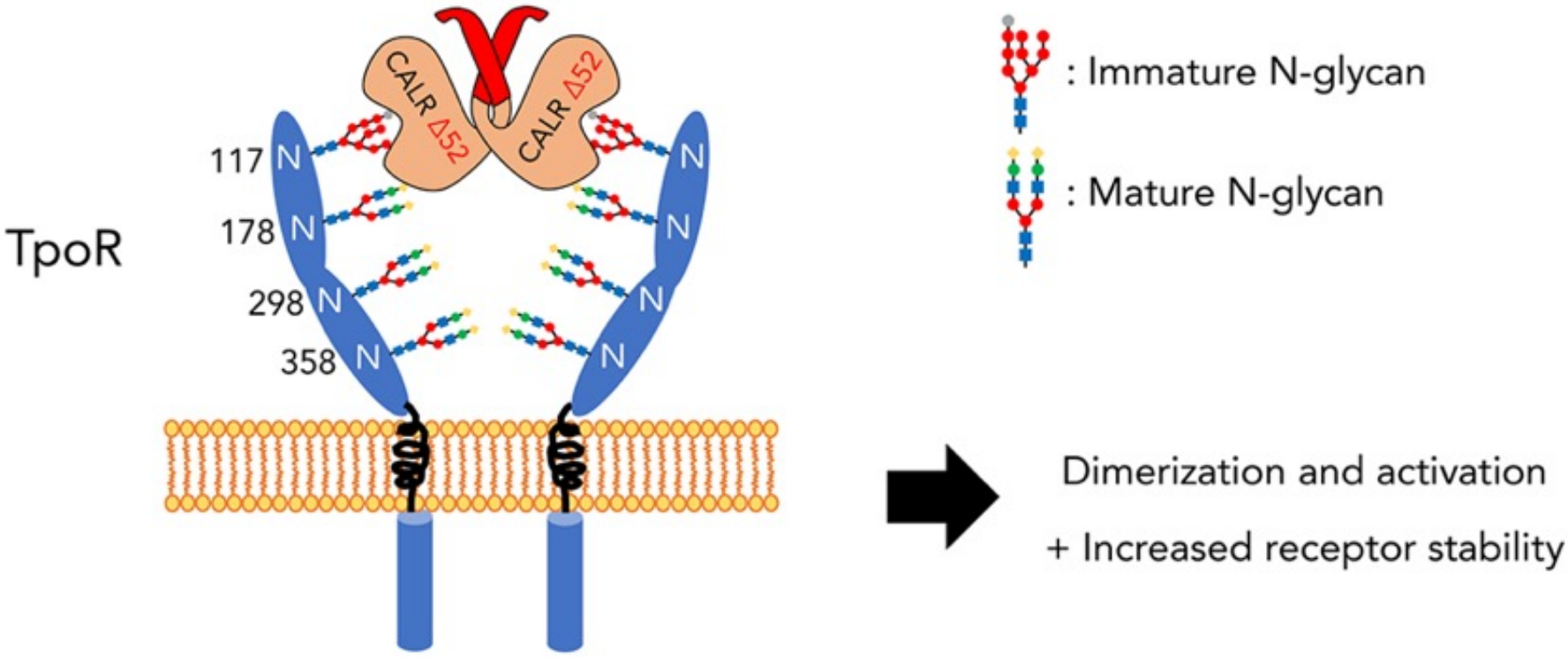
The α-helical of the mutant tail is required for dimerization and activation of TpoR





Papadopoulos et al., under review - Nature Communication

CALR del52 binding and activation of human TpoR



Pecquet et al., Blood 2019

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Somatic Mutations of Calreticulin in Myeloproliferative Neoplasms

Thorsten Klampfl, Ph.D., Heinz Gisslinger, M.D., Ashot S. Harutyunyan, M.D., Ph.D., Harini Nivarthi, Ph.D., Elisa Rumi, M.D., Jelena D. Milosevic, M.Sc., Nicole C.C. Them, M.Sc., Tiina Berg, B.Sc., Bettina Gisslinger, M.Sc., Daniela Pietra, Ph.D., Doris Chen, Ph.D., Gregory I. Vladimer, Ph.D., Klaudia Bagienski, M.Sc., Chiara Milanesi, M.Sc., Ilaria Carola Casetti, M.D., Emanuela Sant'Antonio, M.D., Virginia Ferretti, Ph.D., Chiara Elena, M.D., Fiorella Schischlik, M.Sc., Ciara Cleary, M.Sc., Melanie Six, B.Sc., Martin Schalling, M.Sc., Andreas Schönegger, M.Sc., Christoph Bock, Ph.D., Luca Malcovati, M.D., Cristiana Pascutto, Ph.D., Giulio Superti-Furga, Ph.D., Mario Cazzola, M.D., and Robert Kralovics, Ph.D.

Somatic *CALR* Mutations in Myeloproliferative Neoplasms with Nonmutated *JAK2*

J. Nangalia, C.E. Massie, E.J. Baxter, F.L. Nice, G. Gundem, D.C. Wedge, E. Avezov, J. Li, K. Kollmann, D.G. Kent, A. Aziz, A.L. Godfrey, J. Hinton, I. Martincorena, P. Van Loo, A.V. Jones, P. Guglielmelli, P. Tarpey, H.P. Harding, J.D. Fitzpatrick, C.T. Goudie, C.A. Ortmann, S.J. Loughran, K. Raine, D.R. Jones, A.P. Butler, J.W. Teague, S. O'Meara, S. McLaren, M. Bianchi, Y. Silber, D. Dimitropoulou, D. Bloxham, L. Mudie, M. Maddison, B. Robinson, C. Keohane, C. Maclean, K. Hill, K. Orchard, S. Tauro, M.-Q. Du, M. Greaves, D. Bowen, B.J.P. Huntly, C.N. Harrison, N.C.P. Cross, D. Ron, A.M. Vannucchi, E. Papaemmanuil, P.J. Campbell, and A.R. Green

blood

Prepublished online December 23, 2013;
doi:10.1182/blood-2013-11-539098

JAK2 or *CALR* mutation status defines subtypes of essential thrombocythemia with substantially different clinical course and outcomes

Elisa Rumi, Daniela Pietra, Virginia Ferretti, Thorsten Klampfl, Ashot S. Harutyunyan, Jelena D. Milosevic, Nicole C.C. Them, Tiina Berg, Chiara Elena, Ilaria C. Casetti, Chiara Milanesi, Emanuela Sant'Antonio, Marta Bellini, Elena Fugazza, Maria C. Renna, Emanuela Boveri, Cesare Astori, Cristiana Pascutto, Robert Kralovics and Mario Cazzola

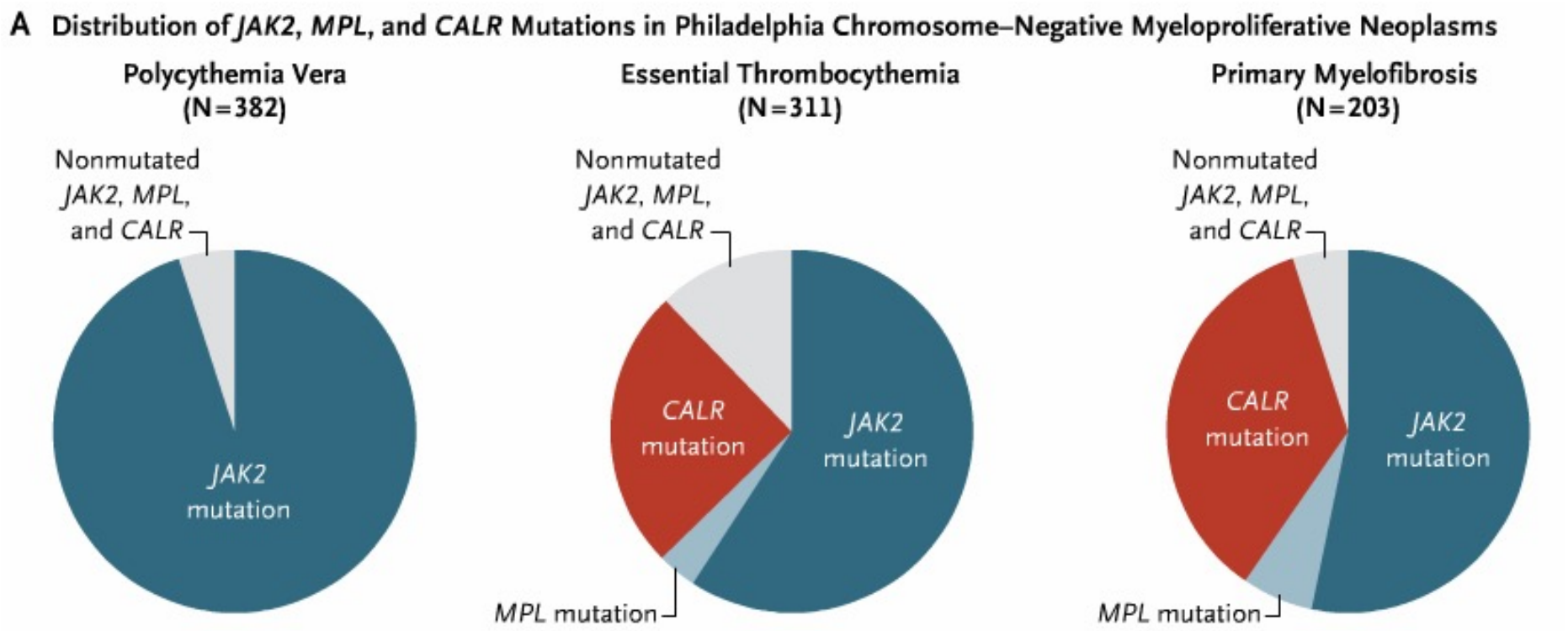
Calreticulin exon 9 frame-shift mutations in patients with thrombocytosis OPEN

J Chi, K Nicolaou, V Nicolaidou, L Kouma, A Mitsidou, C Pierides, M Manoloukos, K Barbouti, F Melanthiou, C Prokopiou, G S Vassiliou, P A Costeas

CALR vs JAK2 vs MPL mutated or triple-negative myelofibrosis: clinical, cytogenetic and molecular comparis

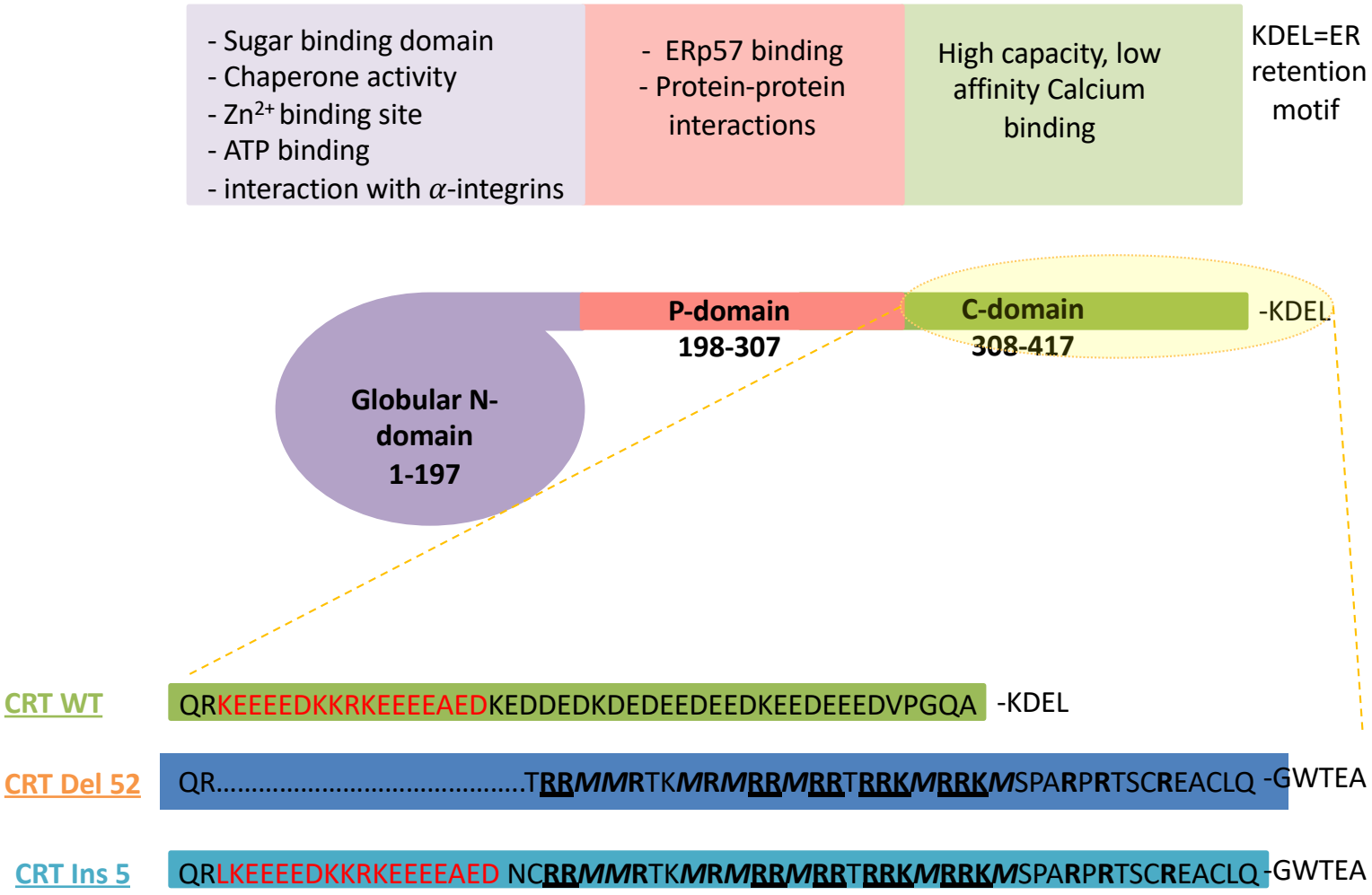
A Tefferi, T L Lasho, C M Finke, R A Knudson, R Ketterling, C H Hanson, M Maffioli, D Caramazza, F Passamonti, A Pardanani

Oncogenic mutations in MPNs



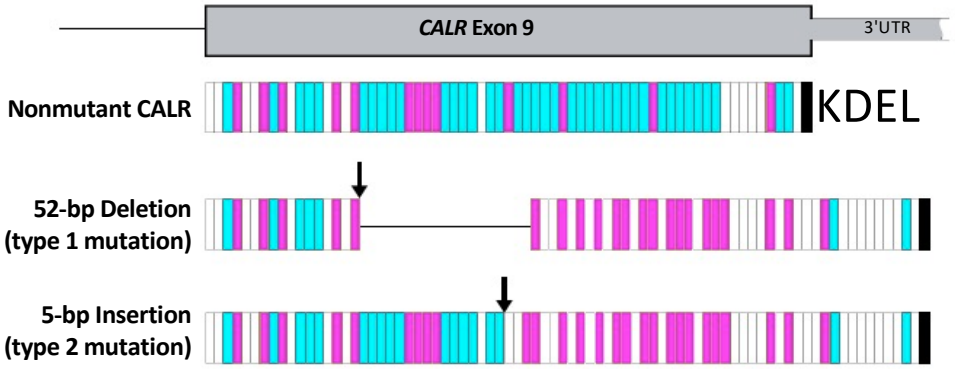
Klampfl et al, NEJM 2013

Klampfl et al. NEJM 2013

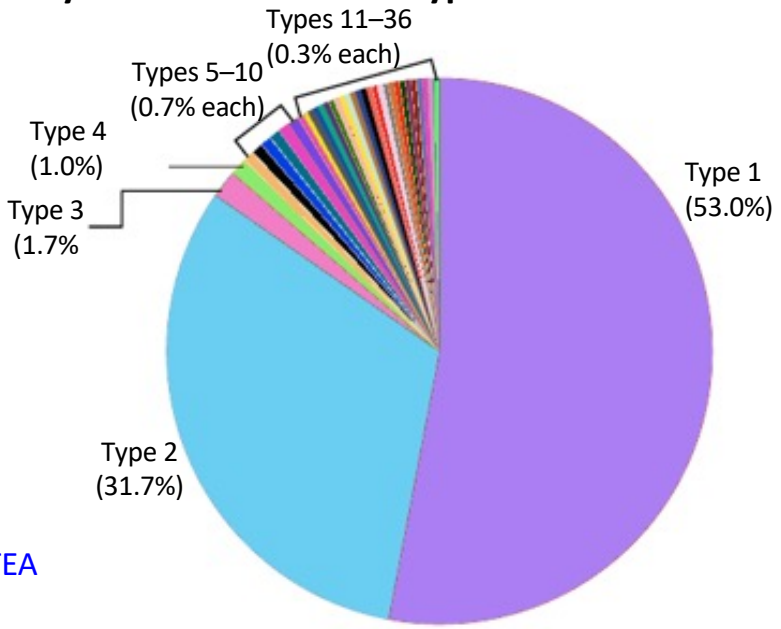

- Sugar binding domain
- Chaperone activity
- Zn2+ binding site
- ATP binding
- interaction with α-integrins
- ERp57 binding
- Protein-protein interactions
High capacity, low affinity Calcium binding
KDEL=ER retention motif
Globular N-domain 1-197
P-domain 198-307
C-domain 308-417
-KDEL
CRT WT
QRKEEEEDKKRKEEEEAEDKEDDEDKDEDEEDEEDKEEDEEEDVPGQA -KDEL
CRT Del 52
QR.....TRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQ -GWTEA
CRT Ins 5
QRLKEEEEDKKRKEEEEAED NCRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQ -GWTEA

CALR Mutations in MPNs

Peptide Sequences of the Two Most Common *CALR* Mutations

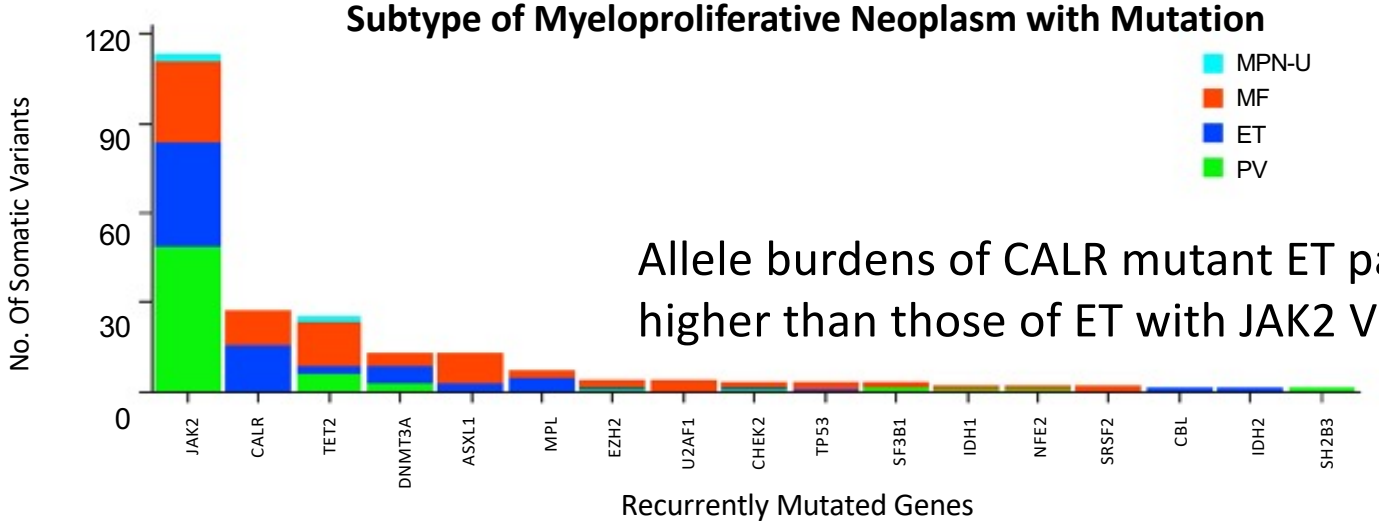


Frequency of the 36 Mutation Types Detected in *CALR*



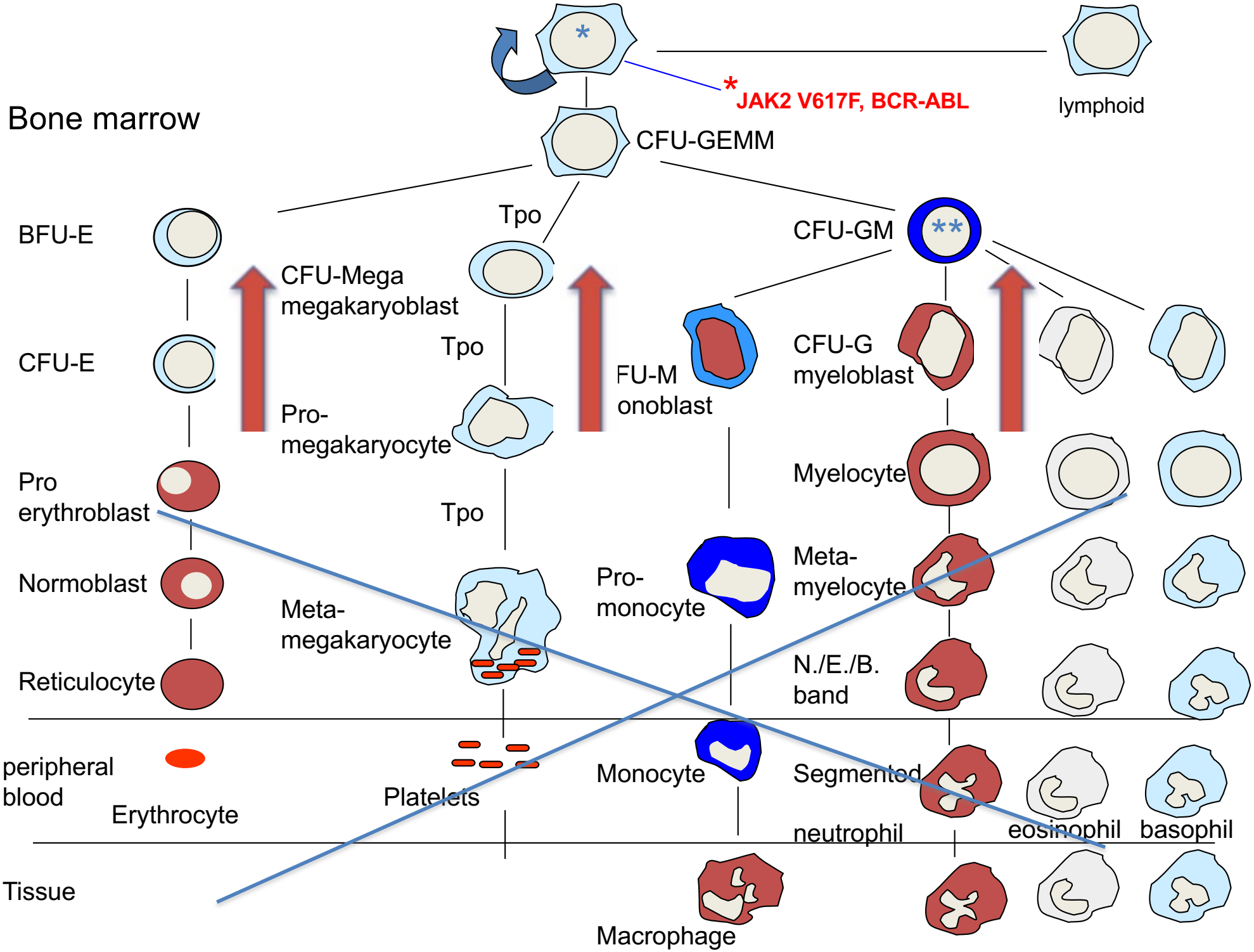
Novel sequence at C-terminus: RRMMRTKMRMRRMMRRKMSPARDPRTSCREACLQGTEA

Subtype of Myeloproliferative Neoplasm with Mutation



Allele burdens of CALR mutant ET patients are significantly higher than those of ET with JAK2 V617F

Klampfl T, et al. N Engl J Med. 2013;369:2379-90, Nangalia J, et al. N Engl J Med. 2013;369:2391-2405.


Bone marrow
*
* JAK2 V617F, BCR-ABL
lymphoid
CFU-GEMM
BFU-E
CFU-E
Pro erythroblast
Normoblast
Reticulocyte
Tpo
CFU-Mega megakaryoblast
Tpo
Pro-megakaryocyte
Tpo
Meta-megakaryocyte
CFU-GM
**
FU-M onoblast
Pro-monocyte
CFU-G myeloblast
Myelocyte
Meta-myelocyte
N./E./B. band
Segmented neutrophil
eosinophil
basophil
peripheral blood
Erythrocyte
Platelets
Monocyte
Tissue
Macrophage

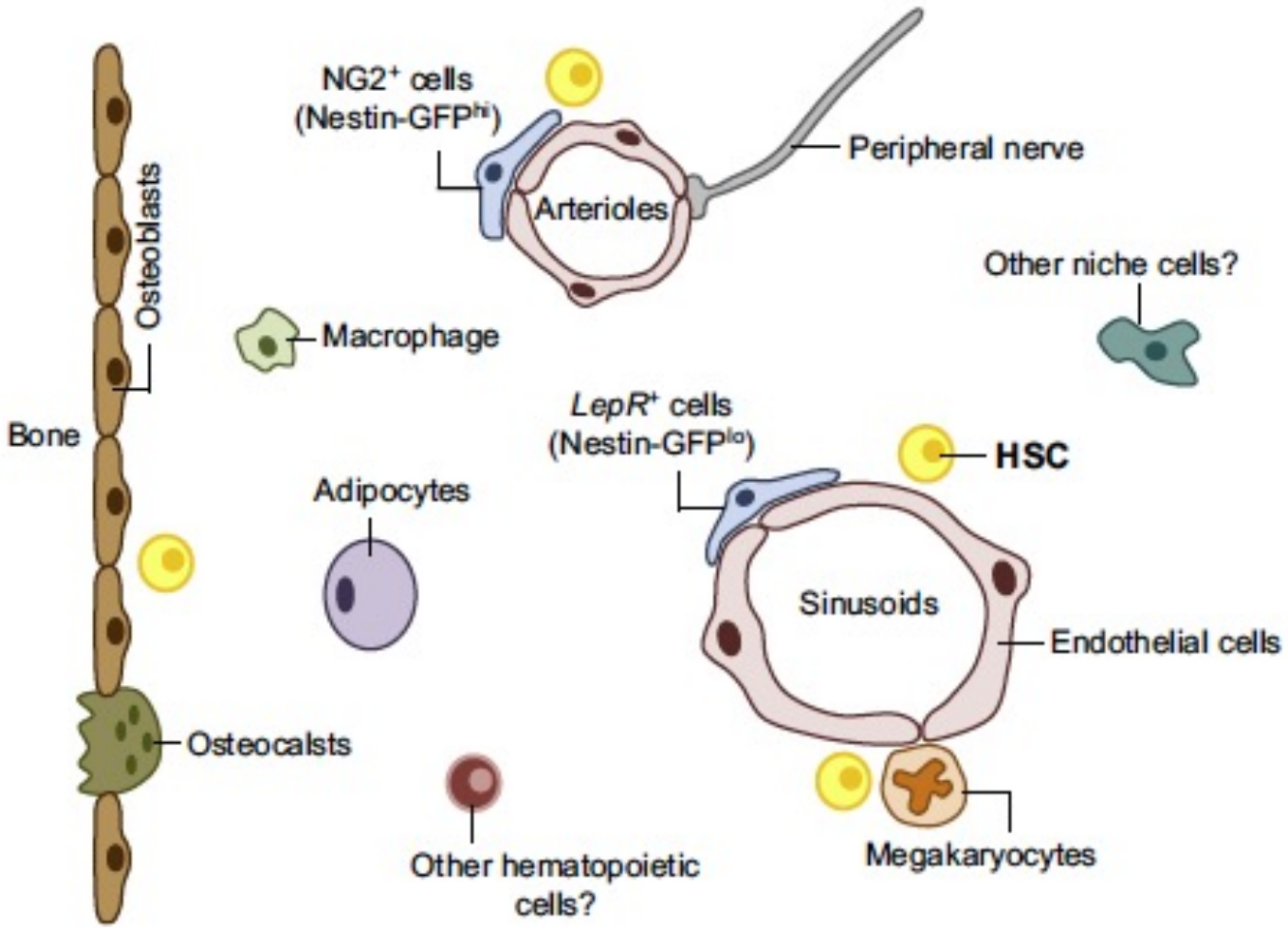
Major clinical problem: the correct choice of transplant time

Too early: 15 % mortality due to transplant (graft versus host)

Too late: 0 % remission, total failure

True for MPNs, MDS, CMML and other mixed MPN/MDS/CMML

Bone marrow hematopoietic stem cell niches



Myeloid progenitors feedback on HSCs and limit cycling; destruction of myeloid cells leads to HSC cyclying

Beerman and Mendez-Ferrer

Experimental Hematology 2017;50:22–26

Are there differences between normal and leukemia stem cells?

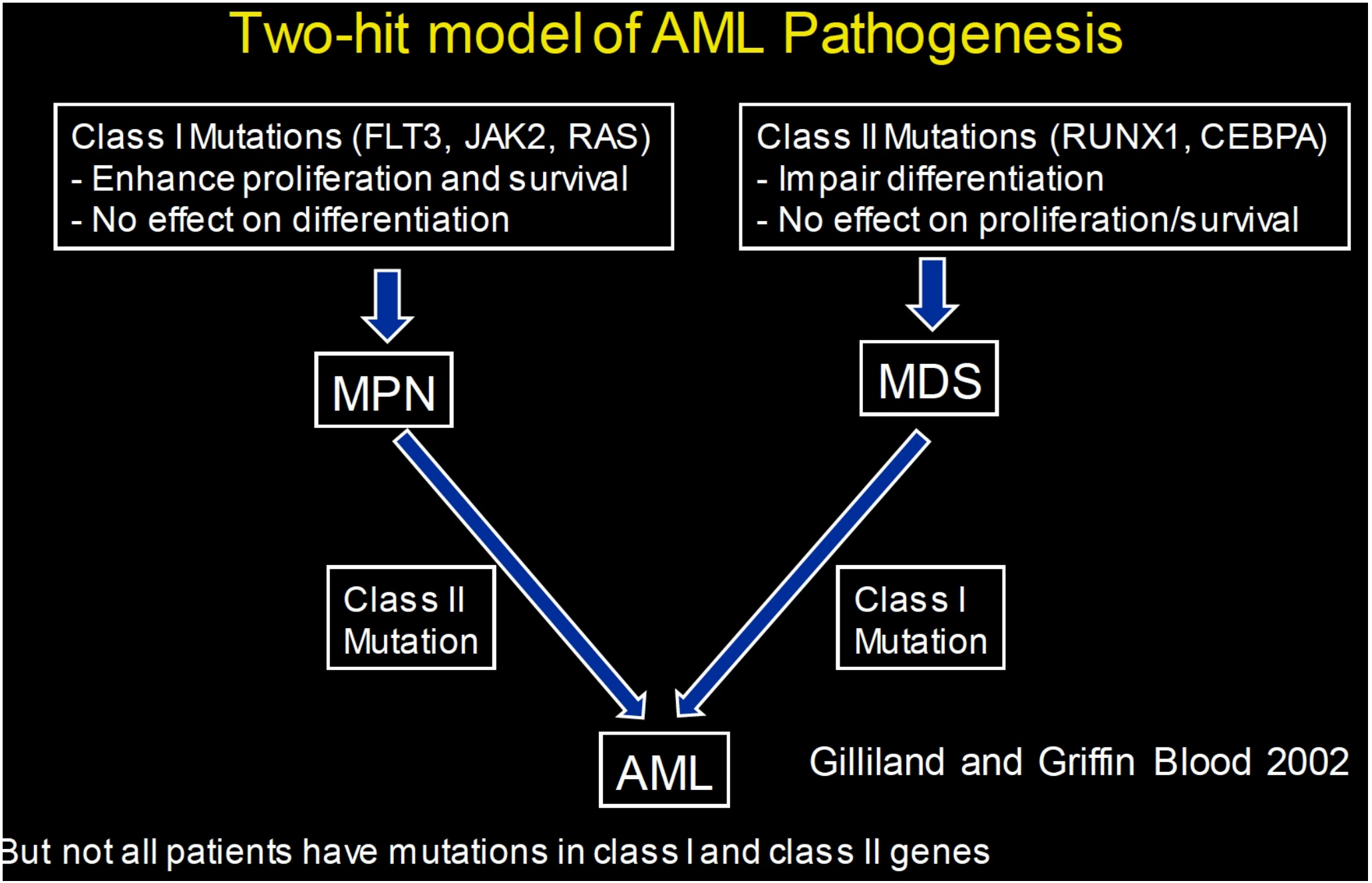
Such as HSCs carrying BCR-ABL in CML/MPN?

Myeloid Stem Cell Markers

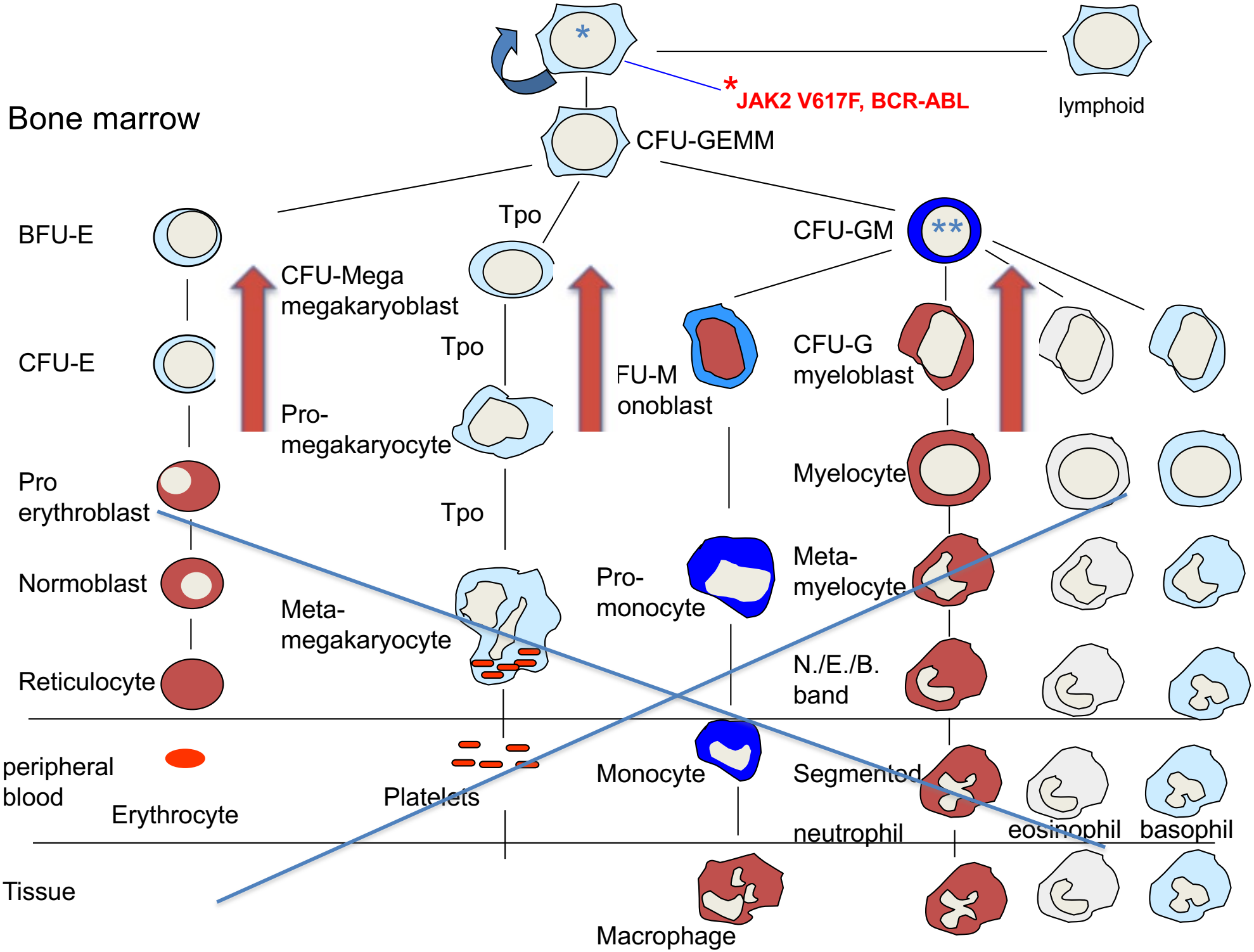
- **Immunophenotype**
- **Lin-CD34+CD38-**
  - **CD90-**
  - **CD123+**
  - **CD99+**
  - **CLL-1+**
  - **IL1RAP+**
  - **CD25+**

Leukemic Stem Cell Antigens

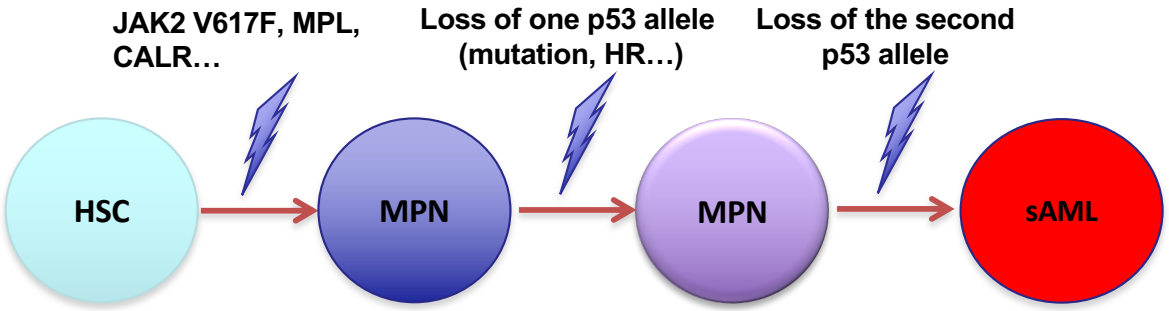
Dr Elisabeth PAIETTA, Educational Session ASH 2012



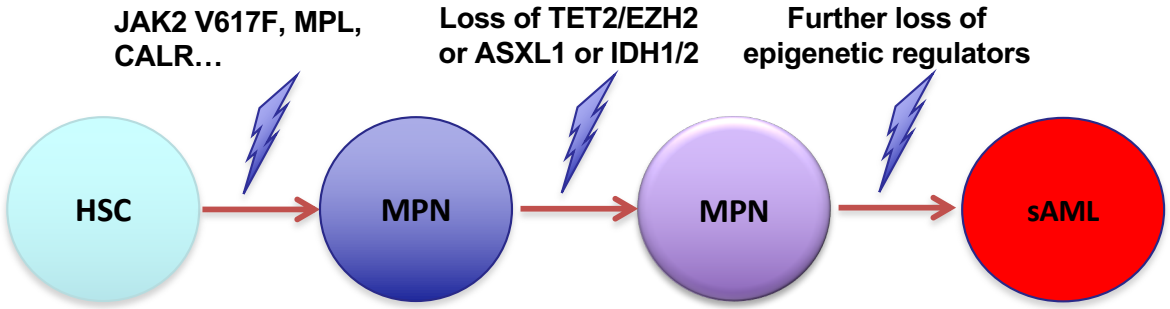
Dr Ross Levine Educational Session ASH 2012, Atlanta


Bone marrow
*
* JAK2 V617F, BCR-ABL
lymphoid
CFU-GEMM
BFU-E
CFU-E
Pro erythroblast
Normoblast
Reticulocyte
Tpo
CFU-Mega megakaryoblast
Tpo
Pro-megakaryocyte
Tpo
Meta-megakaryocyte
CFU-GM
**
FU-M onoblast
Pro-monocyte
CFU-G myeloblast
Myelocyte
Meta-myelocyte
N./E./B. band
Segmented neutrophil
eosinophil
basophil
peripheral blood
Erythrocyte
Platelets
Monocyte
Tissue
Macrophage

MPN Development and Progression Hypotheses



Secondary AML

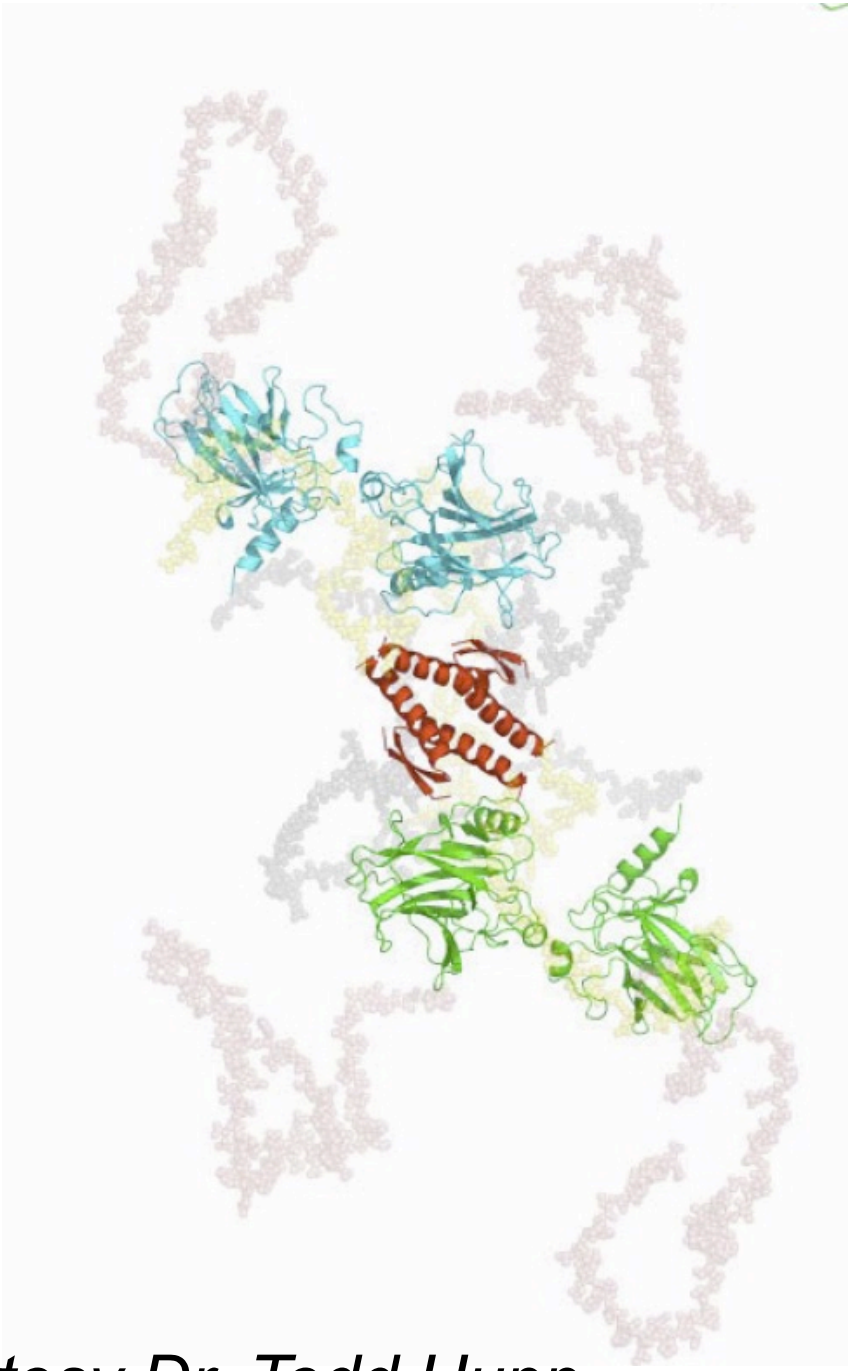


P53 is a tumour suppressor

Key concepts:

The p53 gene is mutated in human cancer

The P53 is “activatable”



Courtesy Dr. Tedd Hupp

P53 in the news

Elephants have 20 copies of a *p53* (or, more properly, *TP53*), in their genome, where humans and other mammals have only one

Peto, R., Roe, F. J., Lee, P. N., Levy, L. & Clack, J. *Br. J. Cancer* **32**, 411–426 (1975).

Abegglen, L. M. *et al*. *J. Am. Med. Assoc.* http://dx.doi.org/10.1001/jama.2015.13134 (2015).

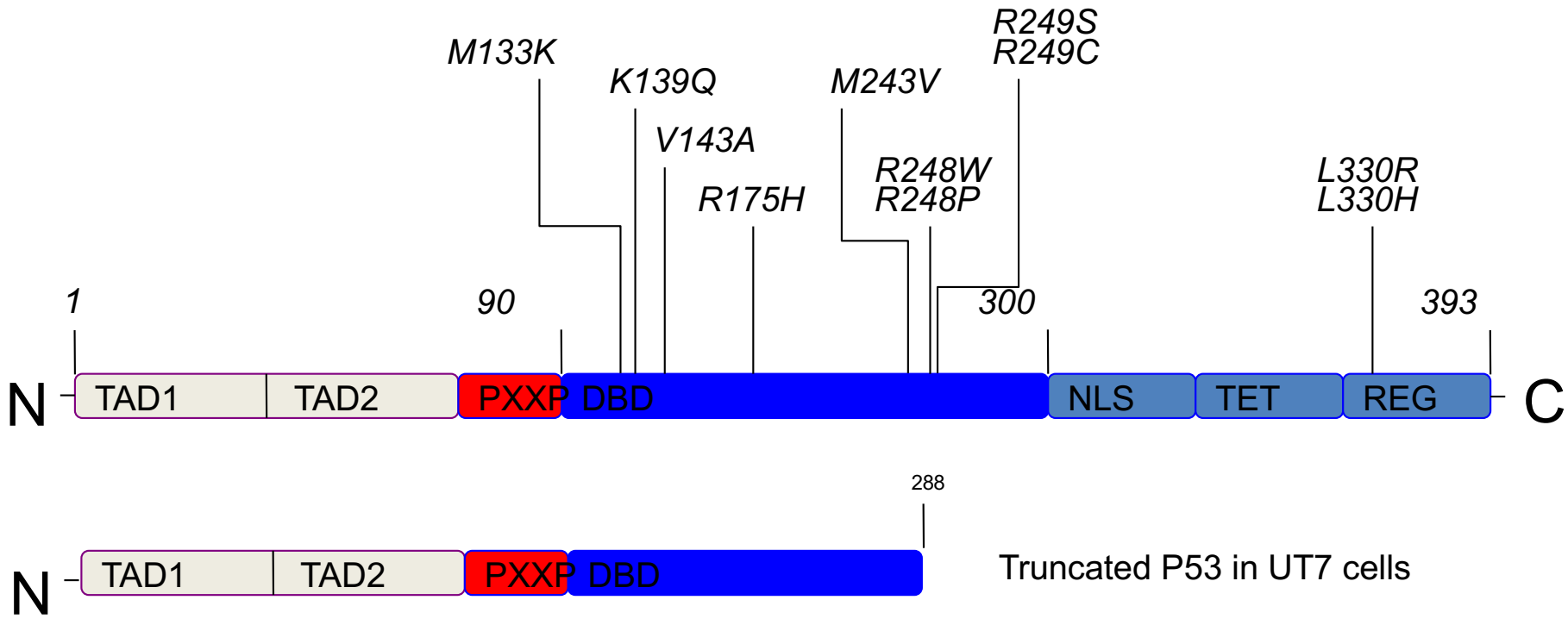
Sulak, M. et al. Preprint at bioRxiv http://dx.doi.org/10.1101/028522 (2015).

More than a dozen *TP53* copies in two extinct species of mammoth, but just one copy in elephants' close living relatives, manatees and hyraxes

Elephant blood cells seem exquisitely sensitive to DNA damage from ionizing radiation

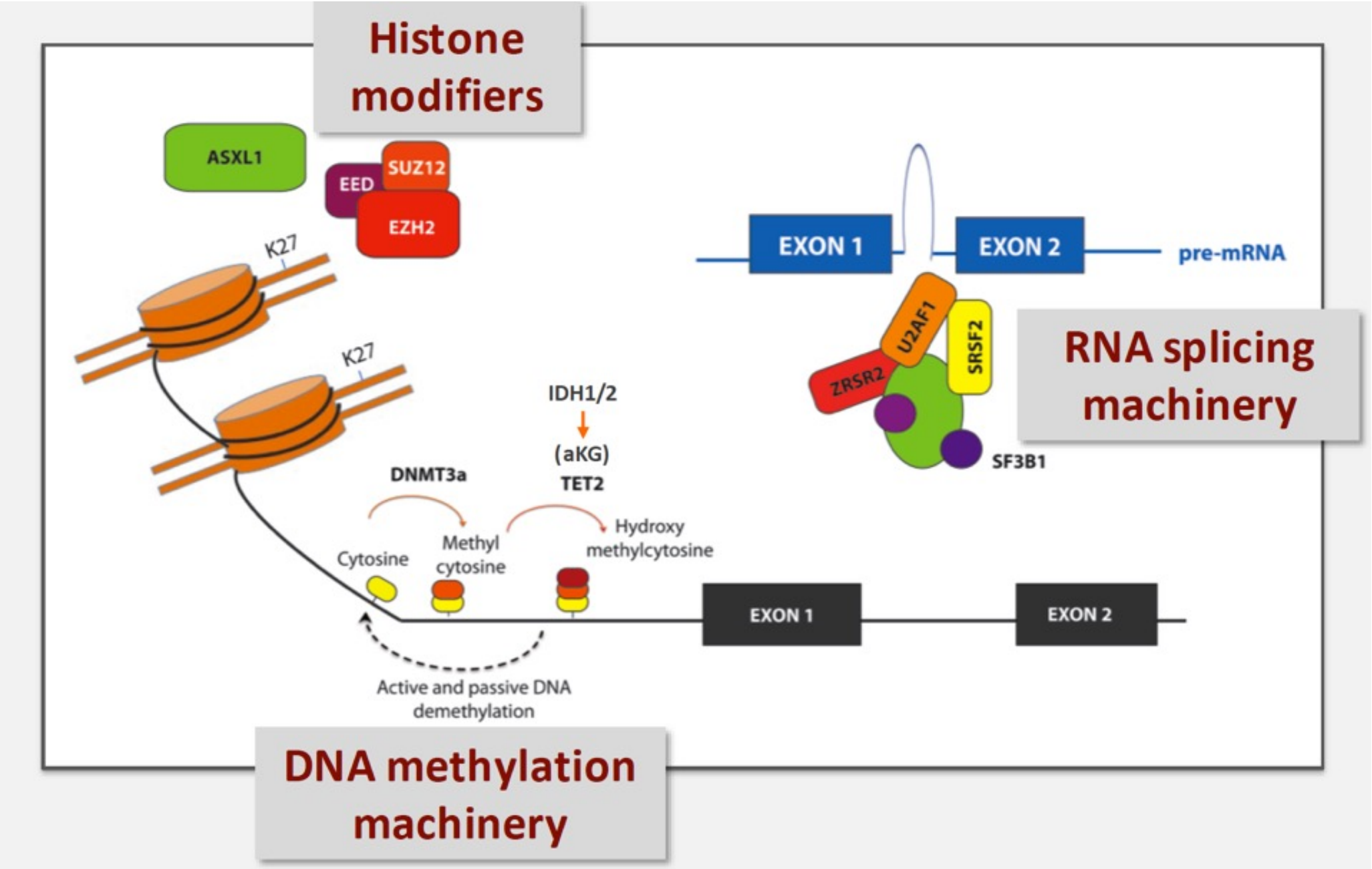
Courtesy Dr. Tedd Hupp

Schematic Representation of p53 Variants



Mutations of TP53 found in many solid tumors end leukemia

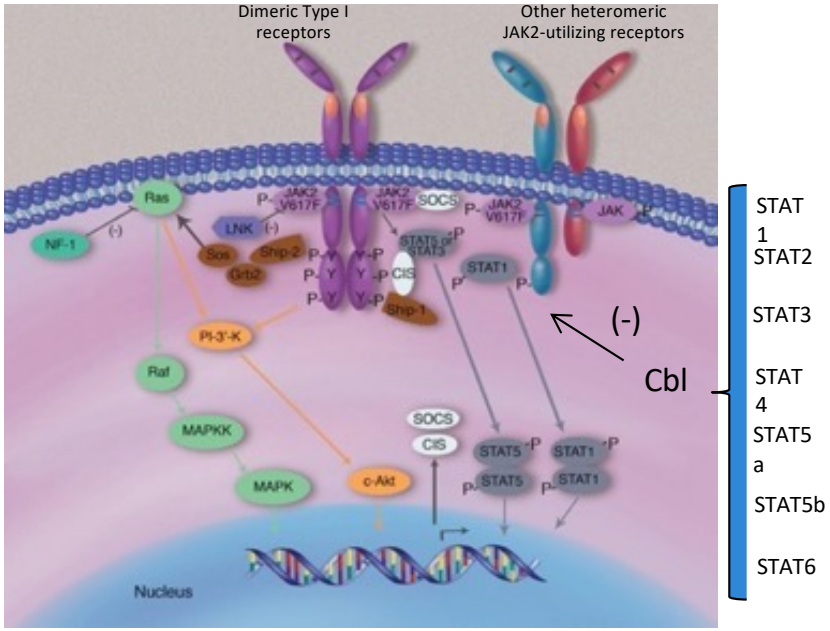
Epigenetic changes in leukemia



Dr Marie Figueroa, Educational Session ASH 2012, Atlanta

Mutations in MPNs

Phenotypic Drivers



Gene	PV[1] (%)	ET[1] (%)	MF[1] (%)	MF[2] (%)
TET2	16	7	9	9.7
IDH1/2	3	0	0	2.6
DNMT3A	3	9	3	5.7
EZH2	2	1	9	5.1
ASXL1	3	1	18	21.7

Clonal dominance and progression

Polycythemia Vera

97%	***JAK2* V617F**
3%	***JAK2* ex 12**

Essential Thrombocythemia

64%	***JAK2* V617F**
4.3%	***MPL* W515X**
<5%	***LNK (SH2B3)***
15.5%	***CALR***
16.1%	**Unknown**

Myelofibrosis (PMF, sMF)

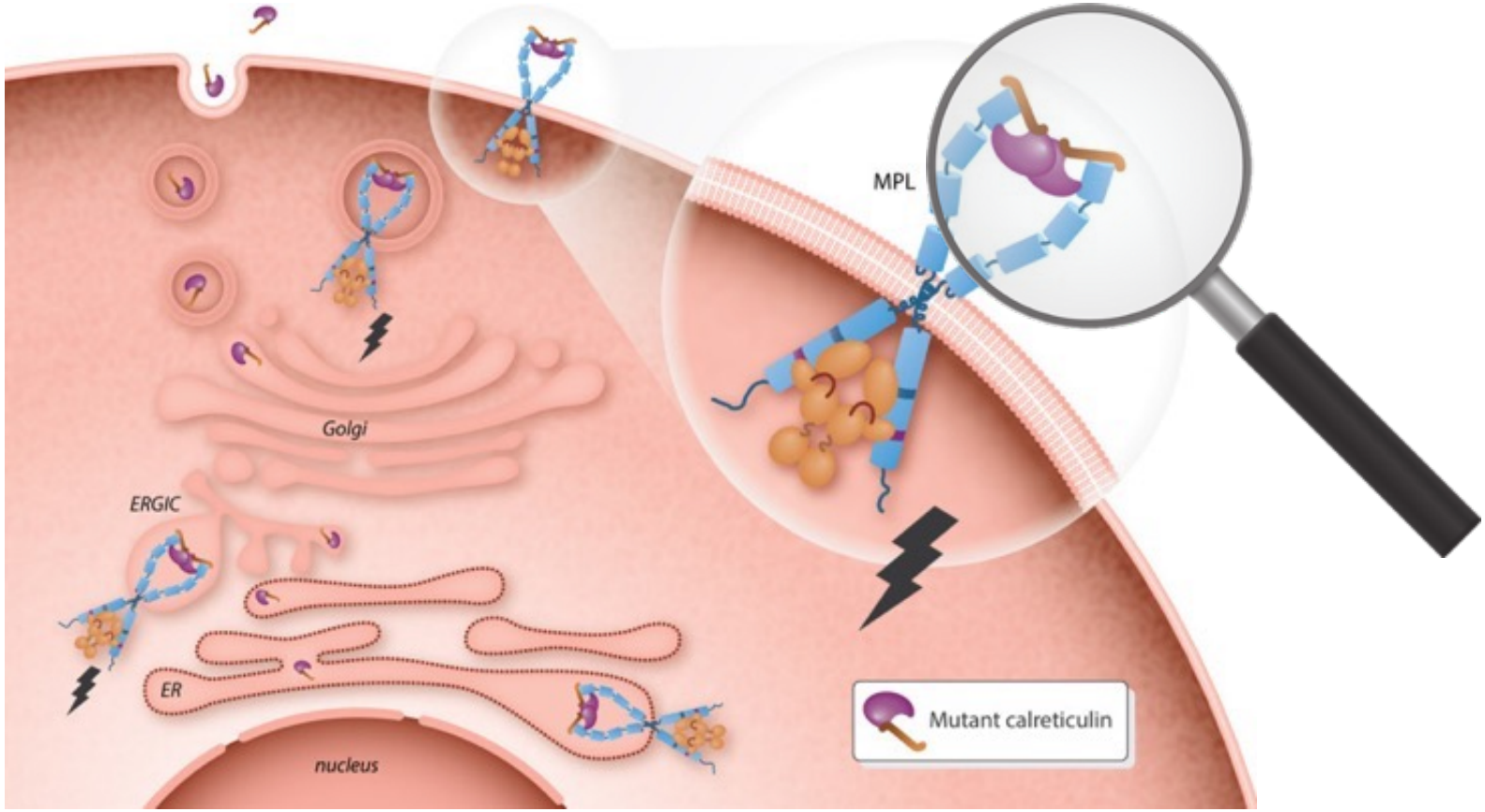
55-64.7%	***JAK2* V617F**
4%	***MPL* W515X**
≤6%	***CBL***
<5%	***LNK (SH2B3)***
22.7-27%	***CALR***
8.6%	**Unknown**

1. Lundberg P et al. Blood. 2014;123:2220-2228.
2. Vannucchi A et al. Leukemia. 2013;27:1861-9.

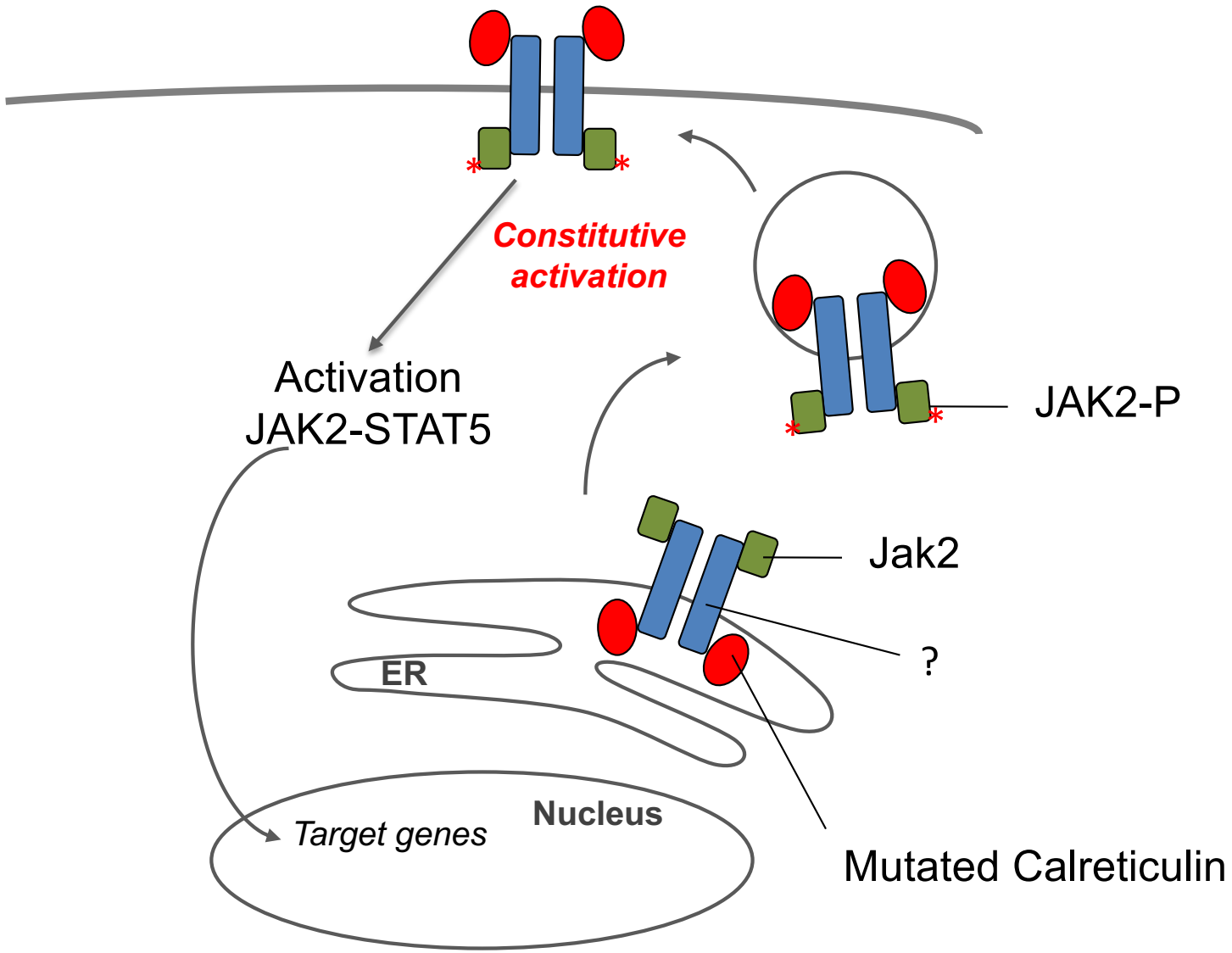
Example of potentially successful treatment

Targeting cell-surface mutant CALR in MPNs and sAML

CALR del52 binding and activation of human TpoR

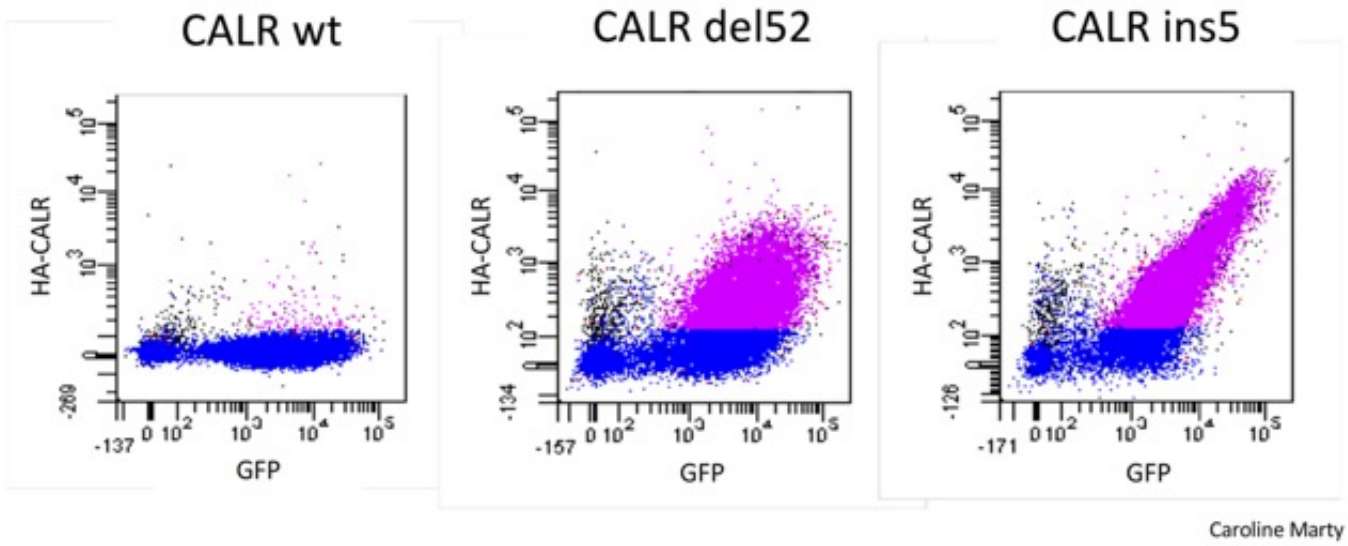


How Can Calreticulin Mutants Induce Myeloproliferative Neoplasms?



Unpublished, Vainchenker W, Kralovics R, Constantinescu SN

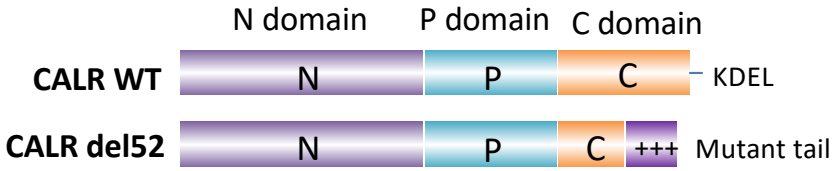
CALR mutants are exposed at the cell surface



Caroline Marty

HA-CALR (IRES-GFP): membrane labeling

CALR del52

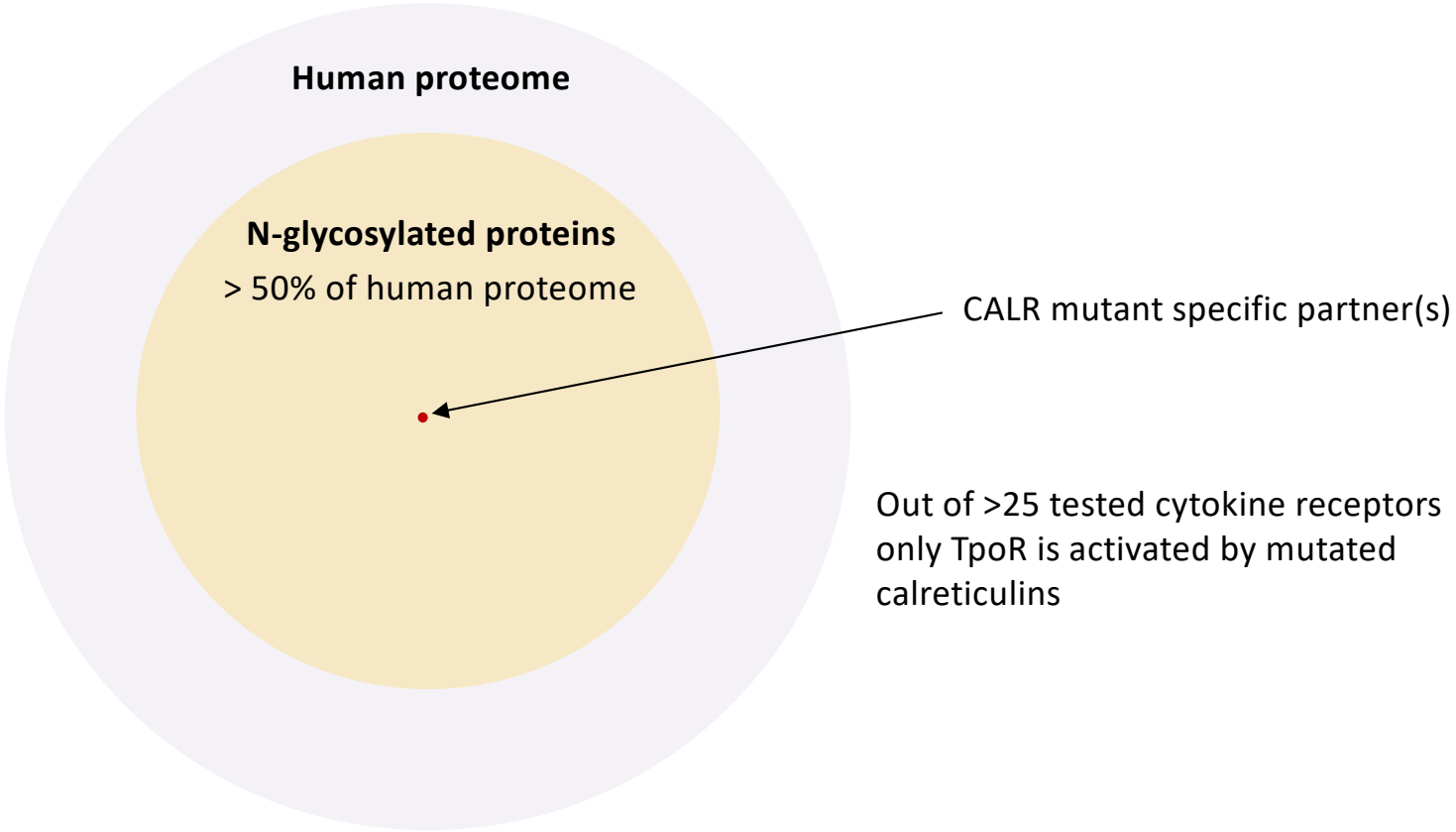


CALR WT …QMKDKQDEEQRLK**EEEED**KKRK**EEEE**A**ED**K**EDDED**K**DEDEEDEED**K**EEDEEED**VPGQA**KDEL

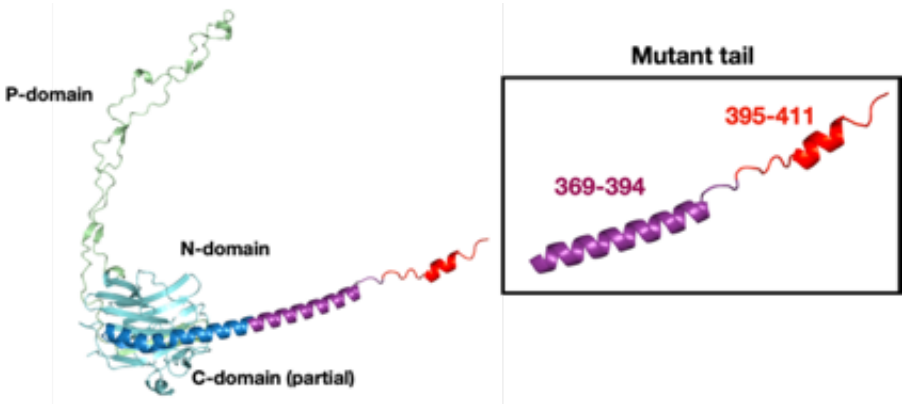
+1 Frameshift

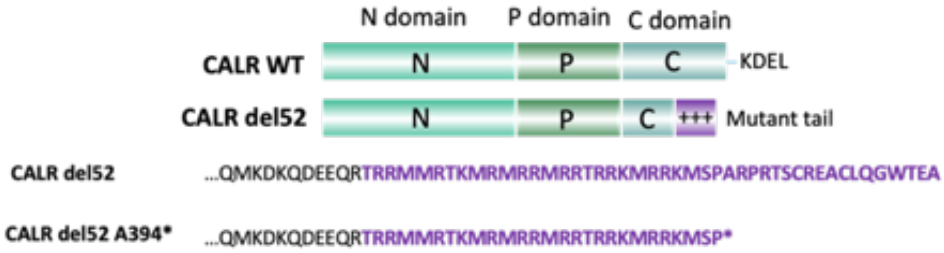
CALR del52 …QMKDKQDEEQRT**RR**MM**R**T**K**M**R**M**RR**M**RR**T**RR**K**M**RR**KMSPA**R**P**R**TSC**R**EACLQGWTEA

Structural Basis of Oncogenesis by Mutants of Calreticulin



The Arg rich segment of the mutant tail is α-helical

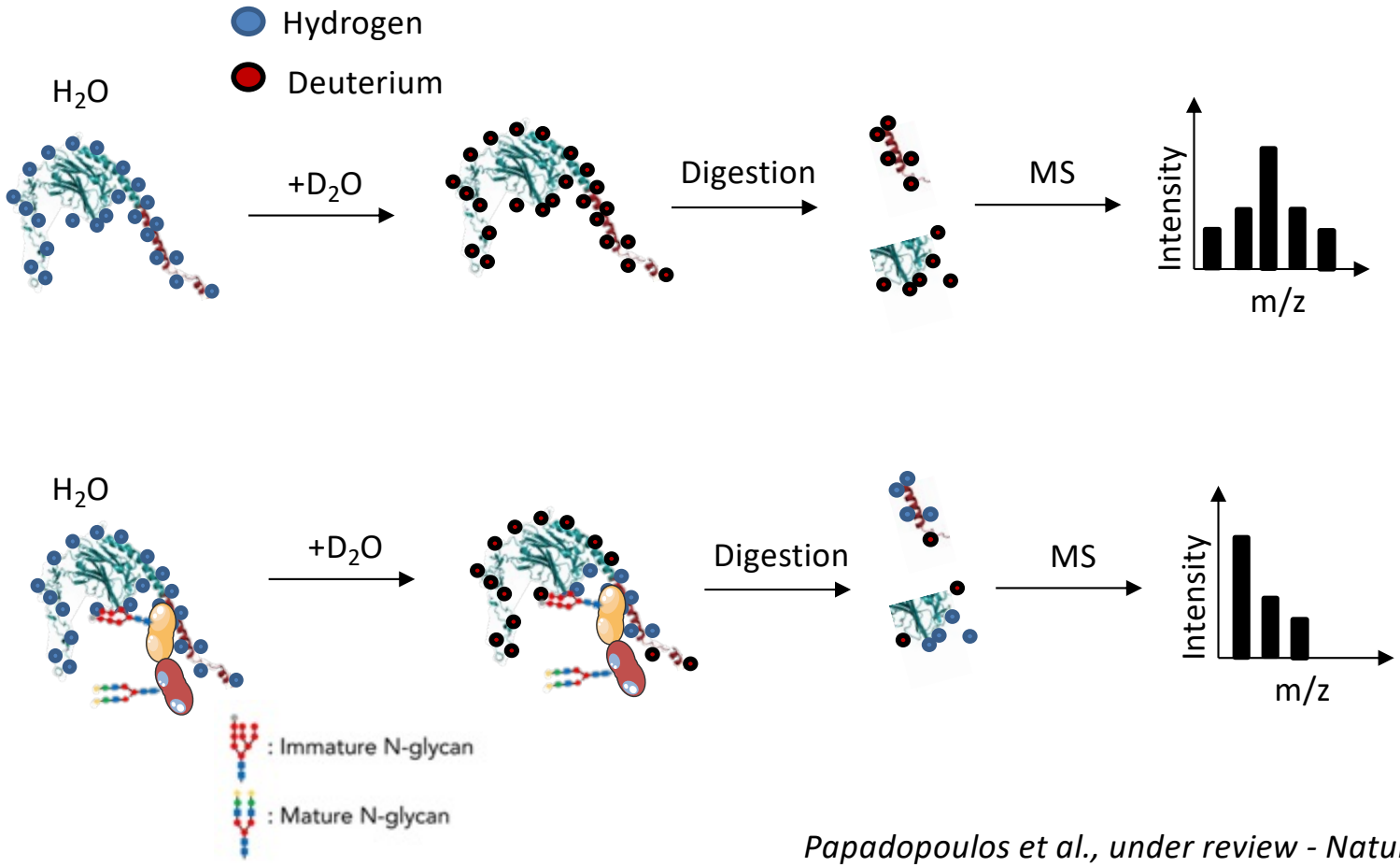




Sample	α-helix(%)	ß-sheet (%)	Turn (%)	Random (%)
CALR WT	13.3 ± 0.9	29.9 ± 0.3	11.6 ± 0.2	40.4 ± 0.5
CALR Δc-tail	0.1 ± 0.2	33.8 ± 0.2	15.4 ± 0.2	44.2 ± 0.3
CALR del52	8.4 ± 0.8	29.6 ± 0.2	13.1 ± 0.1	43.8 ± 0.6
CALR del52 A394*	12.1 ± 0.7	31.5 ± 0.7	12.4 ± 0.3	40.1 ± 40.7

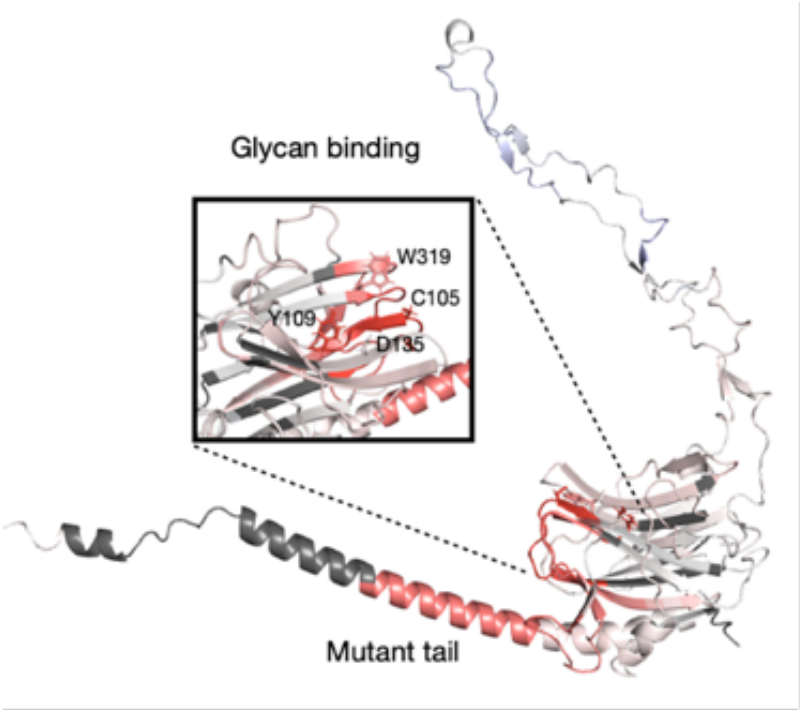
Papadopoulos et al., under review - Nature Communications

HDx-MS mapping of interacting sites between CALR del52 and <u>immature</u> TpoR



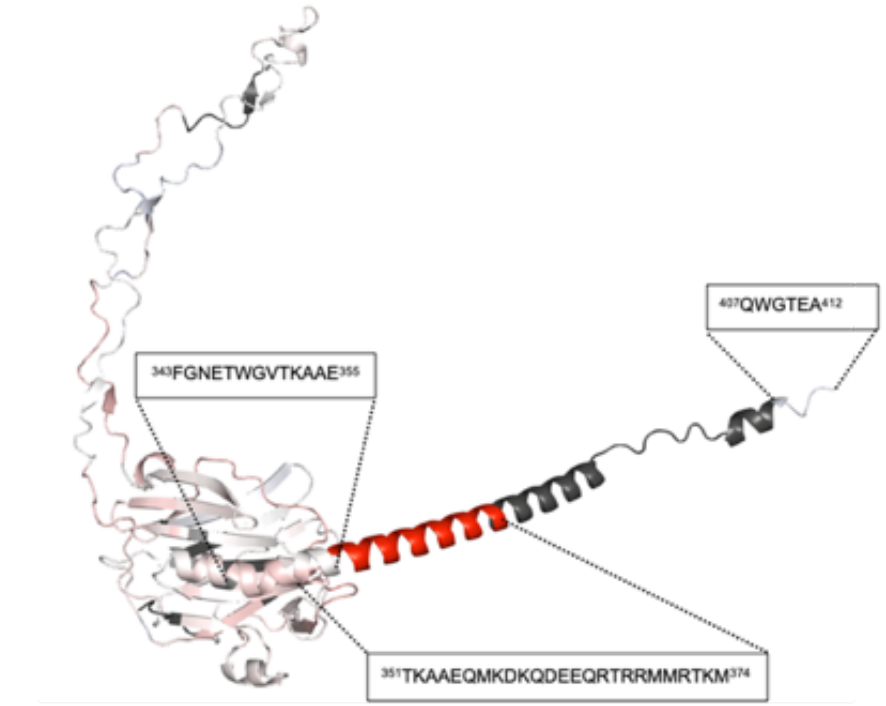
Papadopoulos et al., under review - Nature Communications

HDx-MS mapping of interacting sites between CALR del52 and <u>immature</u> TpoR



Papadopoulos et al., under review - Nature Communication

HDx-MS reveals direct interaction between <u>mature</u> TpoR ECD and CALR mutant tail



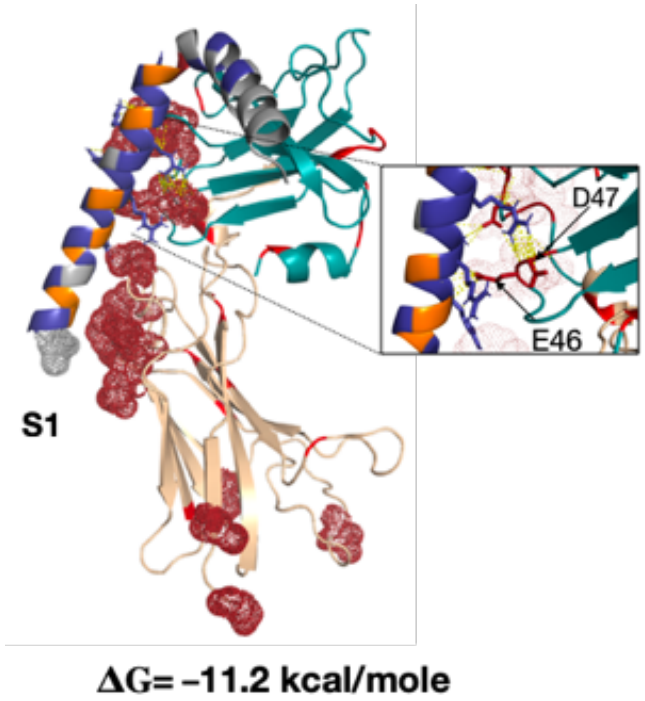
CALR del52 alone vs. CALR del52 + TpoR D1-D4 ECD (mature)

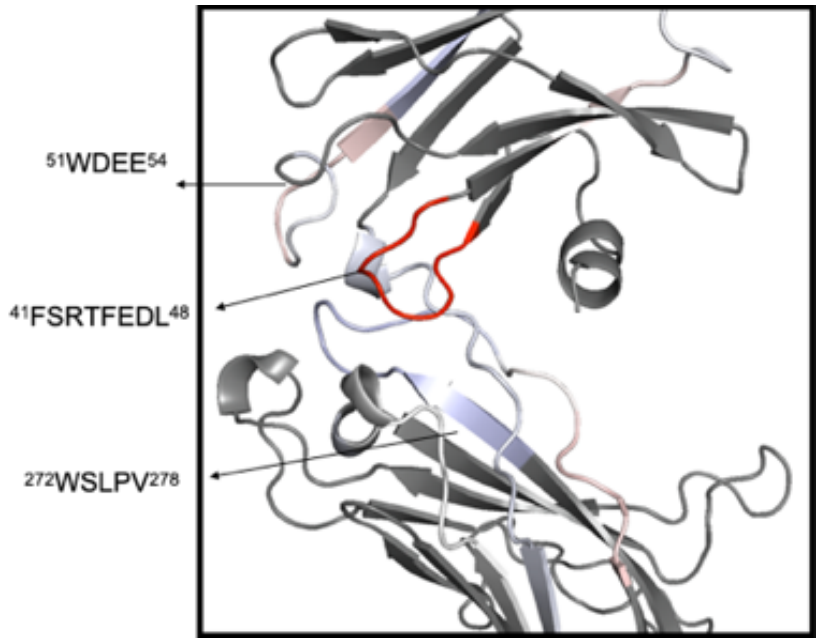
Relative fractional deuterium uptake

Less protected — More protected

Papadopoulos et al., under review - Nature Communications

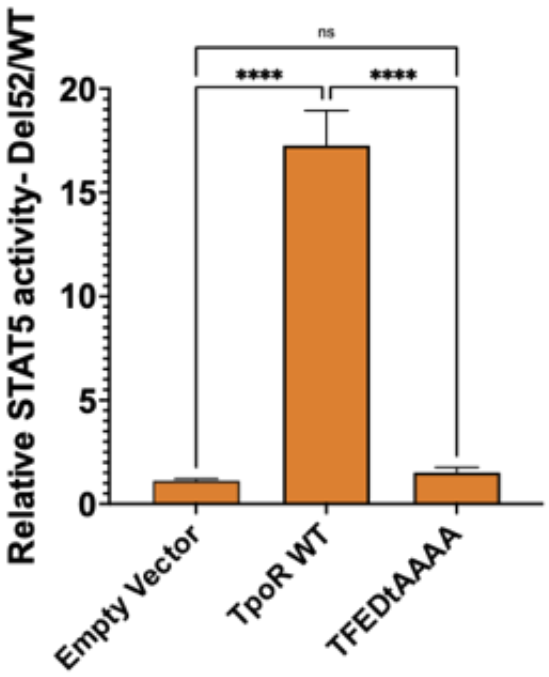
CALR mutant tail interacts with acidic patches on TpoR D1 domain

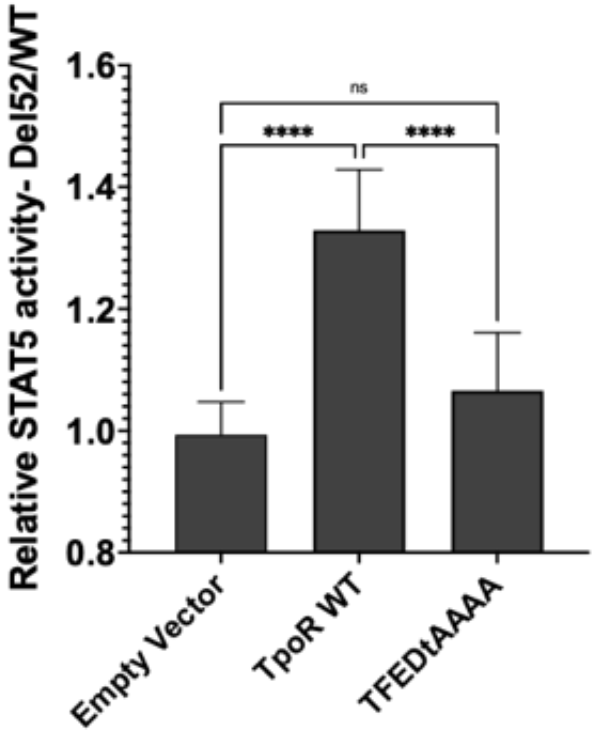




Papadopoulos et al., under review - Nature Communications

The TFED motif is required for TpoR activation by CALR mutant





Papadopoulos et al., under review - Nature Communications

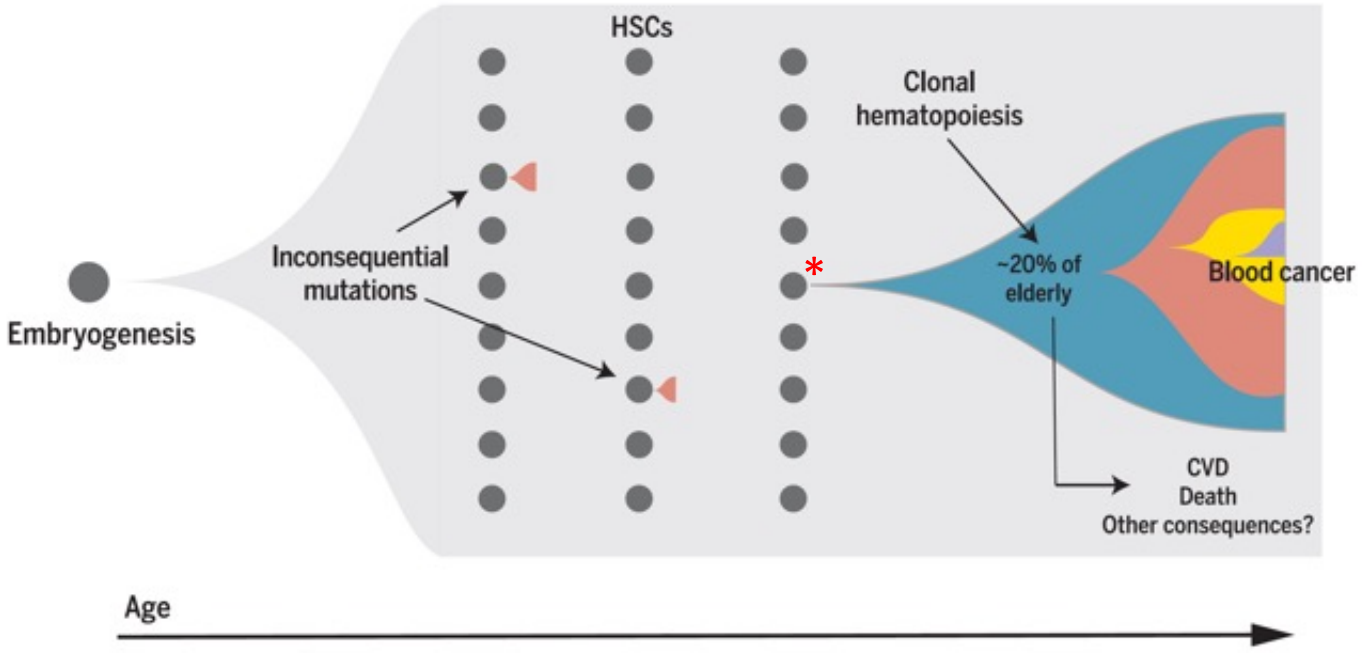
Conclusions

- The specific interaction beteween mutated CALRs and TpoR require both the tail interaction with negative charges in TpoR D1 and the lectin binding domain of CALR forming a complex with immature N-linker sugars on TpoR (N117).
- The charged and helical novel tail of mutated CALRs forms a dimer that induces cross-dimerization of TpoR.
- The interaction of mutated CALRs with TpoR leads dimerization of TpoR transmembrane domains in an active conformation.
- Mutated CALRs act as rogue chaperones for TpoR, exposing immature TpoR at the cell-surface and as rogue cytokines as they activate TpoR.
- The cell-surface accessibility allows one to target mutCALR on the clone and potentially eliminate the clone starting from mutated stem cells.

Clonal Hematopoiesis of Indeterminate Potential

- 2% clonality in peripheral blood without overt blood count anomaly
- Mutations in *DNMT3A*, *TET2*, *ASXL1*, but also *JAK2*, Splicing Factors Genes, *TP53*, *PPMD1*

Hypothesis: CHIP mutations increase fitness of aged Hematopoietic Stem Cell

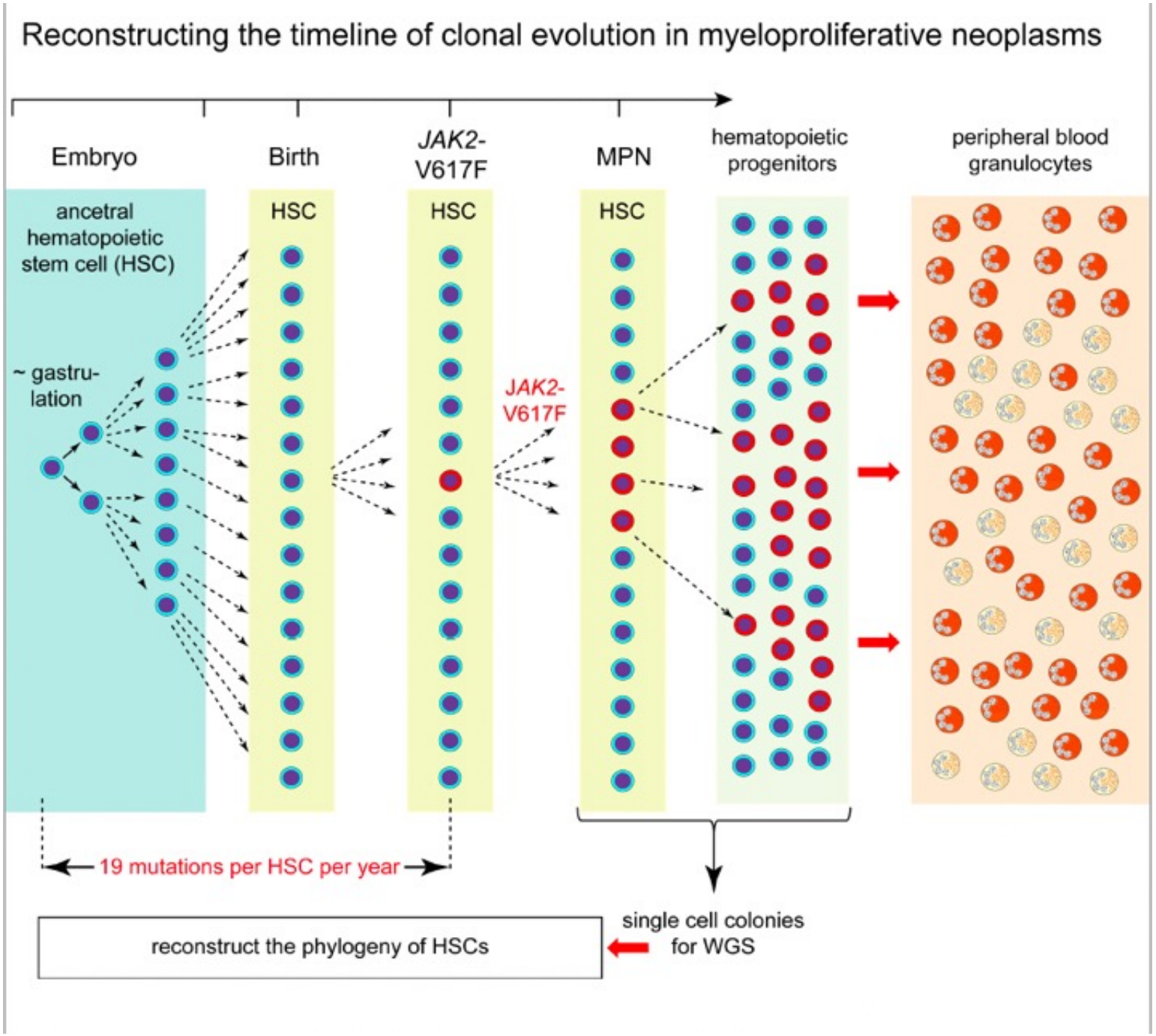


Aside from TP53 and *PPMD1* mutations, *JAK2* mutations are the highest predictors for AML evolution of CHIP individuals

Jaiswal and Ebert, *Science* **366**, 586 (2019) 1 November 2019

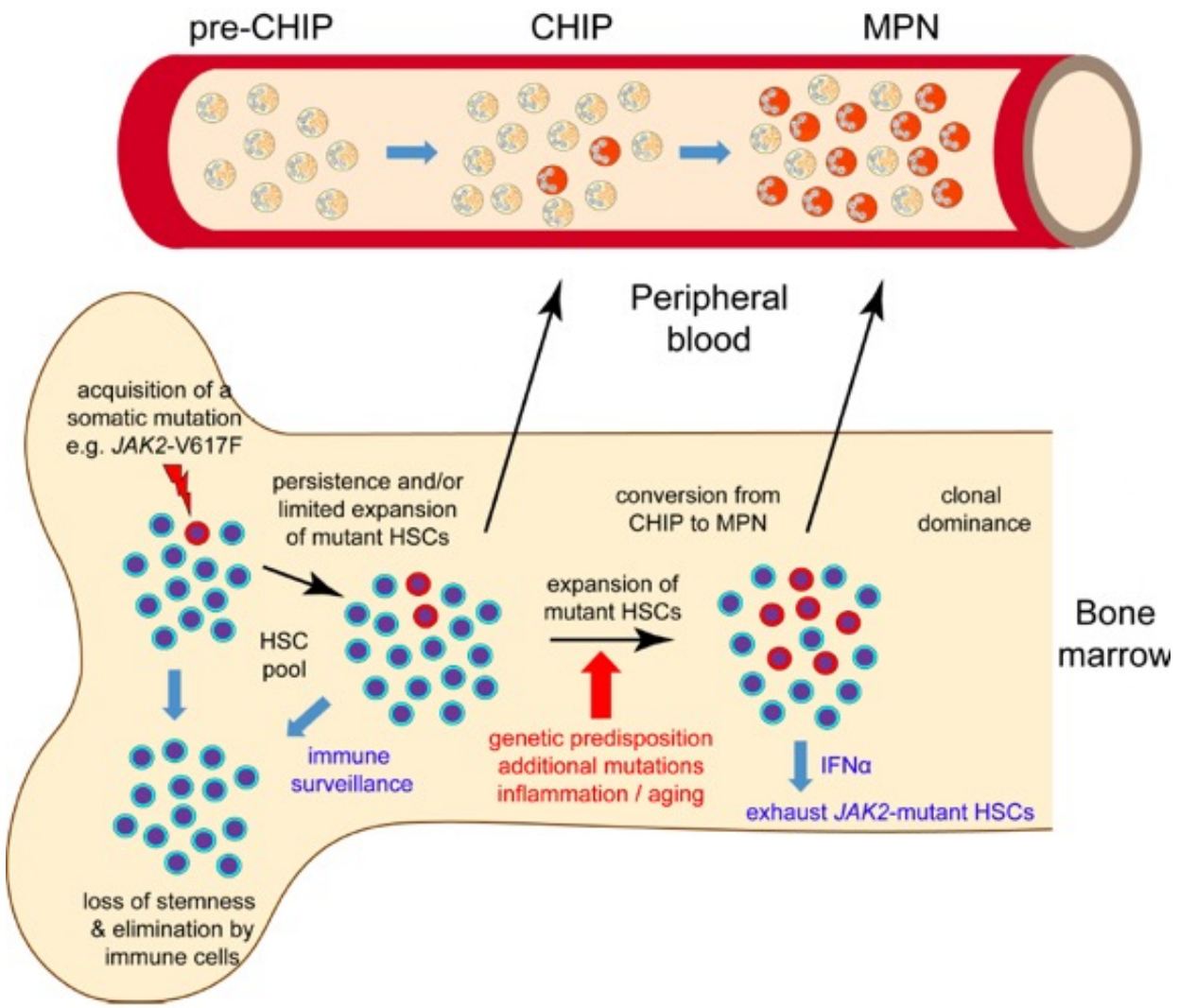
Personalized prevention

- The same mutations that are present in certain MPN, MDS and AML patients can induce earlier in lige CHIP which increases the risk to evolve to those diseases and for vascular diseases, thrombosis and athersclerosis.



Luquez Paz et al. Blood 2022, Nov 8, 200217578

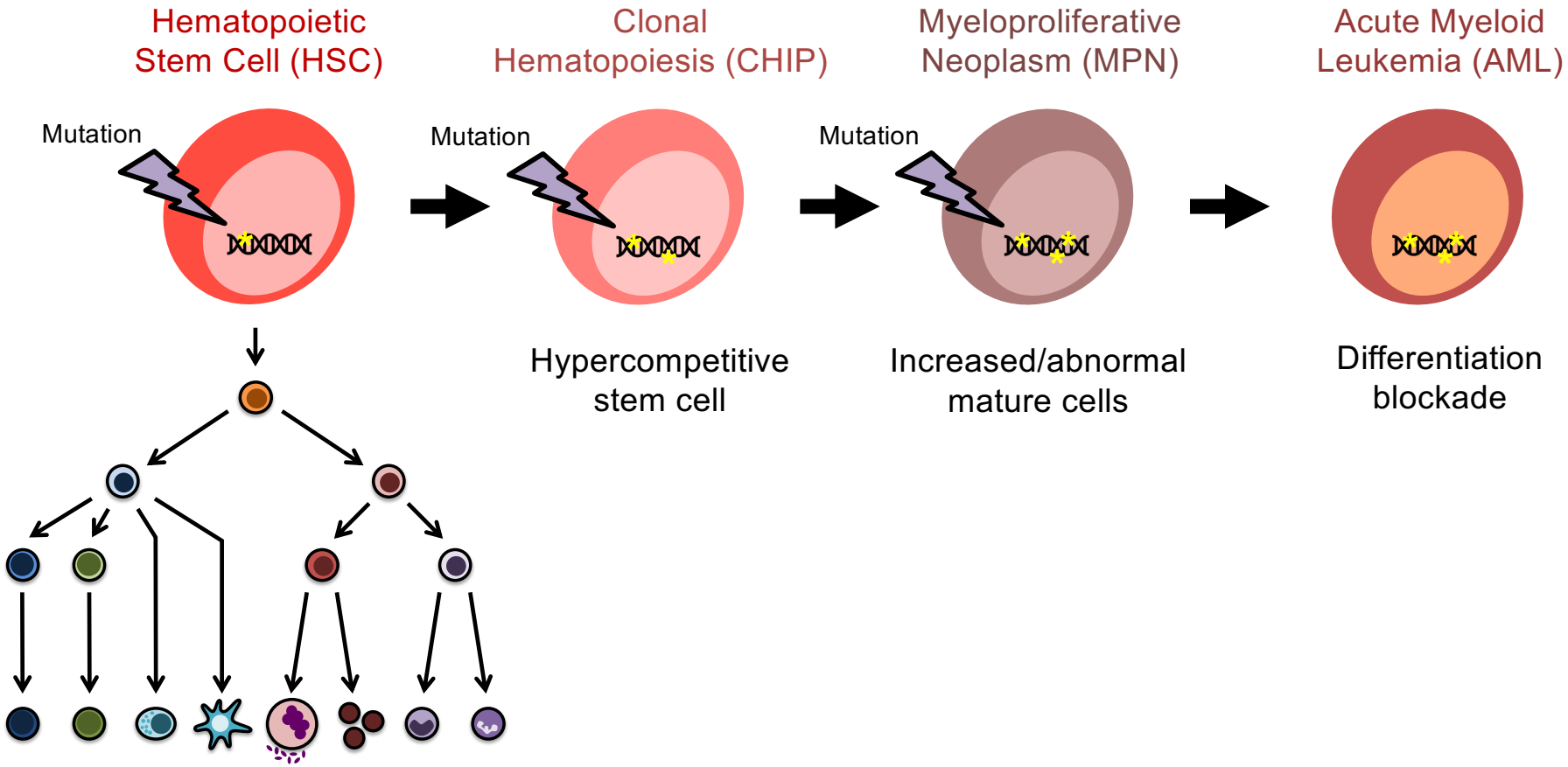
Events from the acquisition of JAK2-p.V617F until the development of MPN.



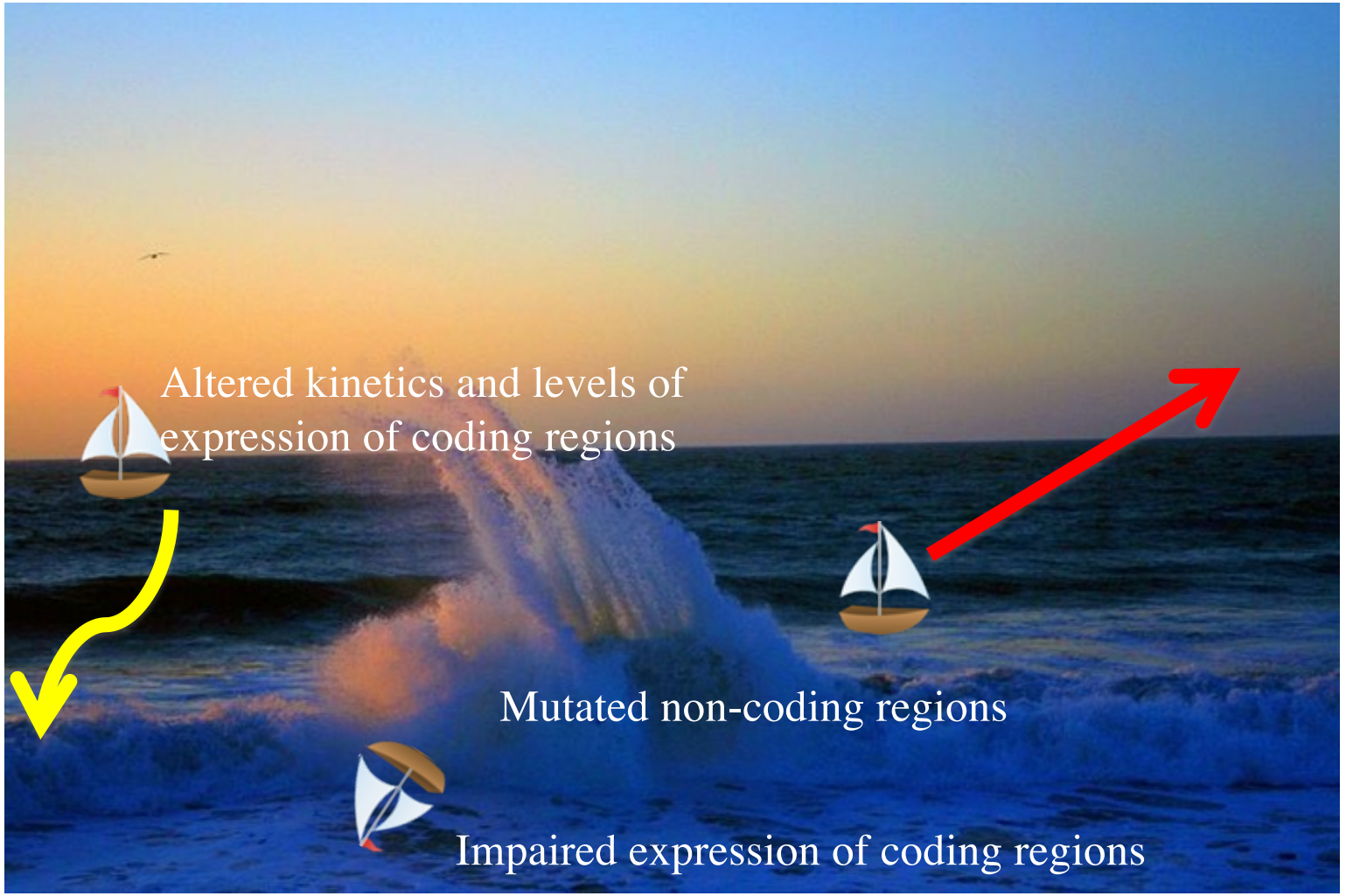
Acquisition of disease driver mutation -) 5-15 years CHIP-) decades MPN

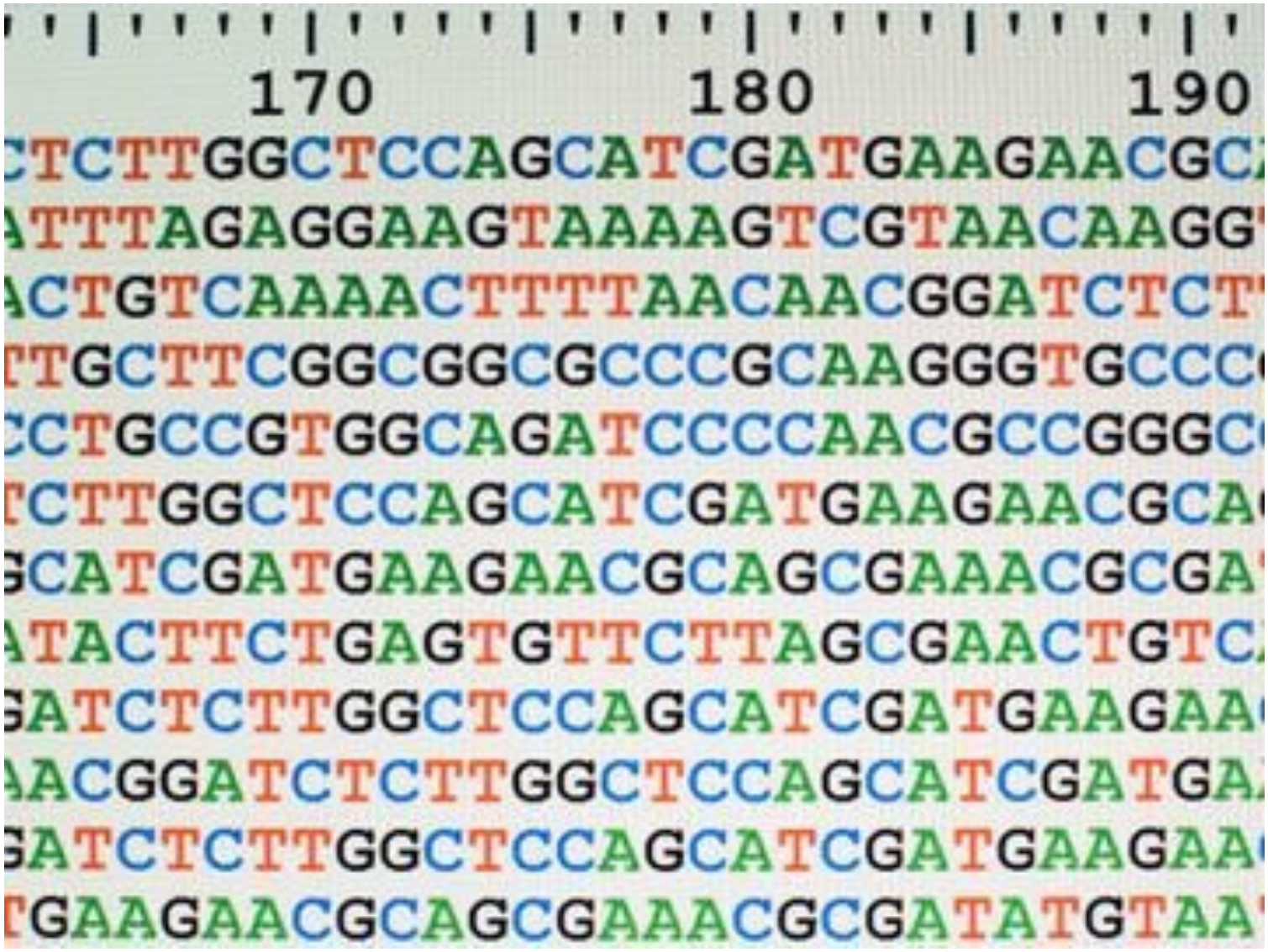
Luquez Paz et al. Blood 2022, Nov 8, 200217578

Myeloid cancer development



N van Gastel

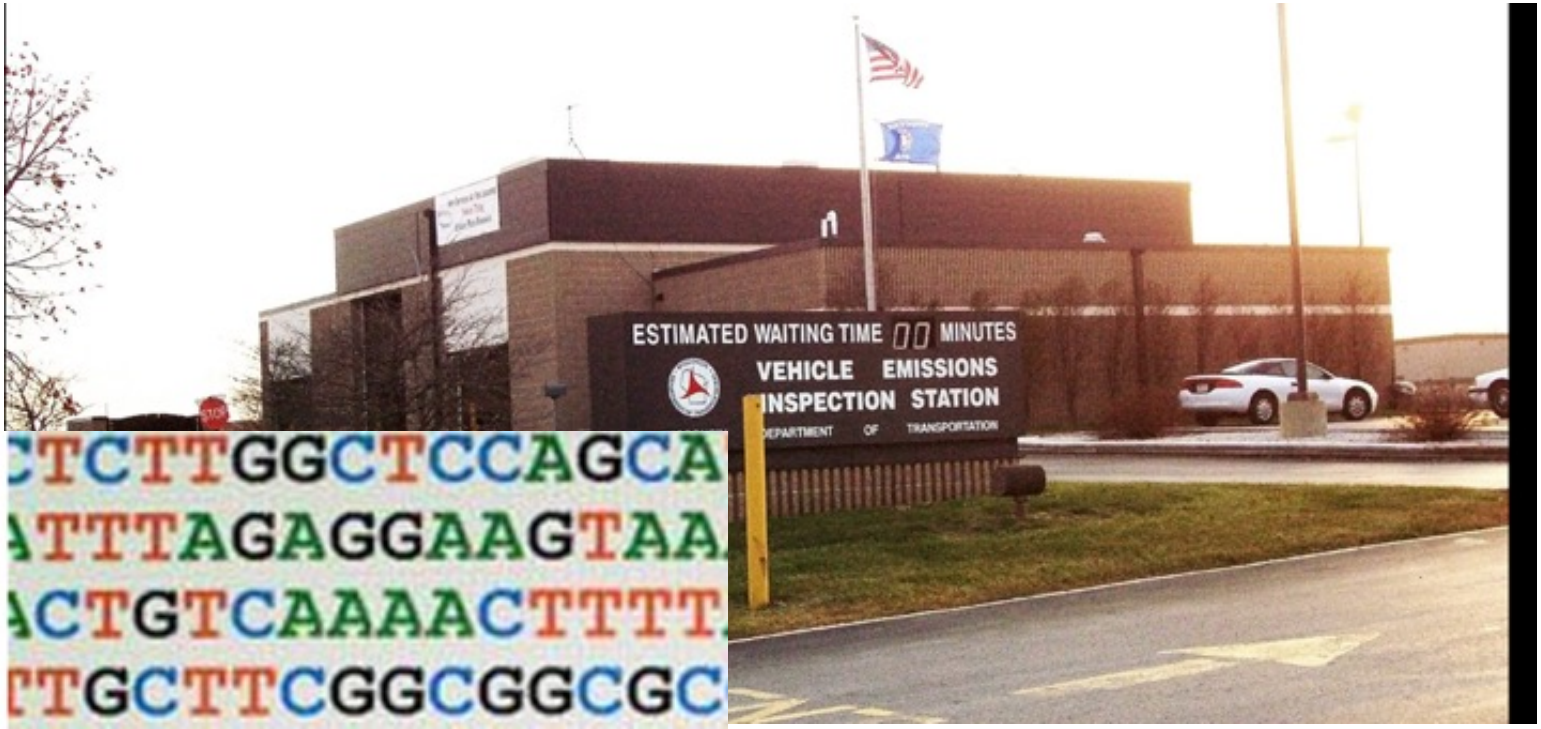

Altered kinetics and levels of
expression of coding regions
Mutated non-coding regions
Impaired expression of coding regions


170
180
190

Guessing The Future.....

“The future is no longer what it isued to be”

Check up for cars, when will we check for early cancer in humans by cell free DNA?



Personalised Medicine, Precision Medicine, Personalised Prevention- within sight?

Acknowledgements

Ludwig Cancer Research, de Duve Institute, Université catholique de Louvain, Brussels, Belgium

Christian Pecquet
Didier Colau
Jean-Philippe Defour
Anita Roy
Emilie Leroy
Ilyas Chachoua
Leila Varghese
Goëlle Vertenoeil
Florian Perrin
Gabriel Levy
Yacine Rahmani
Céline Mouton
Lidvine Genet

de Duve Institute

PHOS and MASS SPEC

Didier Vertommen

de Duve Institute

CELL

Donatienne Tyteca

de Duve Institute & LICR Mouse Facility

Pedro Gomez
Guy Warnier

Ludwig Institute for Cancer Research, Nuffield Department of Medicine, University of Oxford, UK

Xiu Lu
Skirmantas Kriaucionis
Jens Rittscher
Benoît Van den Eynde
Yang Shi

Research Center for Molecular Medicine Of the Austrian Academy of Sciences Vienna, Austria

Robert Kralovics
Harini Nivarthi

Juntendo University School of Medicine Tokyo, Japan

Norio Komatsu
Yoshihiko Kihara
Yoshitaka Sunami
Yoko Edahiro
Marito Araki

MyeloPro GmBH Vienna, Austria

Erica Xu
Eva Hug
Rouchen Ji

Institut Gustave Roussy INSERM U1170 Villejuif, France

William Vainchenker
Isabelle Plo
Caroline Marty

National Institute of Pathology Victor Babes Bucharest, Romania

Emmanuel Fertig
Alina Alexandru

University of Pavia Pavia, Italy

Ilaria Carola Casetti
Elisa Rumi

Institute of Biochemistry Romanian Academy

Andrei-José Petrescu
Toedor Sulea

Medical University of Vienna, Vienna, Austria

Heinz Gisslinger
Bettina Gisslinger
Martin Schalling

SUNY Stony Brook

Steven O. Smith

Standford University

K. Christopher Garcia
Ignacio Moraga

WIMM, University of Oxford

Paresh Vyas
Irene Roberts

Ludwig Institute, San Diego Small molecule Group

Andy Shiau
Tim Gahmann

ARC Project UCLouvain Saint-Luc Hospital

Violaine Havelange
Jean-Philippe Defour
Pascale Saussoy
Dominique Latinne
Carlos Graux
Jean-Baptiste Demoulin

Université de Montpellier, Nîmes

Serge Carillo

University Hospital of Nîmes

Benjamin Papoular

Hôpital Saint-Louis, APHP, Paris

Bruno Cassinat

IRCCS Policlinico San Matteo Foundation, Pavia, Italy

Daniela Pietra
Chiara Cavalloni
Luca Arcaini
Mario Cazzola

Salus Sanguinis

Fondation contre le Cancer

Les avions de Sébastien