



Importància de la biologia molecular en el diagnòstic de les NMP Ph-negatives

Beatriz Bellosillo
Hospital del Mar, Barcelona

Clasificación de la OMS - 2008

- Leucemia mieloide crónica

- Policitemia Vera

- Trombocitemia Esencial

- Mielofibrosis Primaria

Ph neg

Clásicas

- Leucemia neutrofílica crónica

- Leucemia eosinofílica crónica

- Mastocitosis

- Neoplasias mieloproliferativas no clasificables

- **Mutaciones adquiridas**
 - *JAK2*
 - *MPL*
 - Epigenética y *splicing*
 - *CALR*

Marcadores moleculares en NMPs

Citogenética

JAK2V617F

Clonalidad PICX

Cultivos

PRV-1
NFE2

JAK2 exon 12
MPL(W515;S505)

1970-1990

2000

2005

2008-2011

2013

Mutación V617F JAK2

THE NEW ENGLAND JOURNAL OF MEDICINE

ARTICLE

Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis

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Letters to nature

A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera

Chloé James^{1*}, Valérie Ugo^{2,3,4*}, Jean-Pierre Le Couëdic^{1,4}, Judith Staerk¹, François Delhommeau^{1,4}, Catherine Lacout¹, Laïc Garçon¹, Hana Raslova¹, Roland Berger¹, Anne-Isse Bennaceur-Grissolle^{1,4}, Jean-Luc Villevial¹, Stefan N. Constantinescu¹, Nicole Casadevall^{1,5} & William Vainchenker^{1,7}

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⁶Laboratoire d'Hématologie, Institut Gustave Roussy, 94805 Villejuif Cedex, France

⁷Polycythémie d'Hématologie, Hôpital Saint Louis, AP-HP, 75475 Paris Cedex 10, France

⁸Genetics

⁹Genetics

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JH2 pseudo-kinase domain of the Janus kinase 2 (JAK2) gene in most (>80%) polycythaemia vera patients. The mutation, a valine-to-phenylalanine substitution at amino acid position 617, leads to constitutive tyrosine phosphorylation activity that promotes cytokine hypersensitivity and induces erythrocytosis in a mouse model. As this mutation is also found in other myeloproliferative disorders, this unique mutation will permit a new molecular classification of these disorders and novel therapeutic approaches.

In polycythaemia vera, the mechanisms leading to erythropoietin hypersensitivity and *in vitro* production of erythroid colonies in the absence of cytokines (referred to hereafter as EEC, for endogenous erythroid colonies)⁸ are still unknown. We previously reported that inhibitors of JAK2 (AG490), phosphatidylinositol-3-OH kinase (PI(3)K) and Src pathways hampered spontaneous erythroid terminal differentiation in polycythaemia vera⁹. We then focused on JAK2, an upstream molecule directly linked to erythropoietin receptor (EpoR) signalling⁷. In a first set of experiments, we used a short interfering RNA (siRNA) to knockdown JAK2 expression. This siRNA, in contrast to a control siRNA, decreased JAK2 protein levels to less than 10% in UT7 cells (Fig. 1a). It also impaired spontaneous erythroid differentiation in cells from polycythaemia vera patients, markedly inhibited EEC formation (Fig. 1b and c, left panel) and inhibited by 50% erythropoietin-dependent erythroid colony formation in polycythaemia vera cells (Fig. 1c, middle panel) and a normal control (Fig. 1c, right panel).

ORIGINAL ARTICLE

A Gain-of-Function Mutation of JAK2 in Myeloproliferative Disorders

Robert Kralovics, Ph.D., Francesco Passamonti, M.D., Andreas S. Buser, M.D., Soon-Siong Teo, B.S., Ralph Tiedt, Ph.D., Jakob R. Passweg, M.D., Andre Tichelli, M.D., Mario Cazzola, M.D., and Radek C. Skoda, M.D.

Mechanisms of Disease

Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders

Lancet 2005; 365: 1054–61 E Joanna Baxter¹, Linda M Scott¹, Peter J Campbell¹, Clare East, Nicos Fourouclas, Sheila Swanton, George Vassiliou, Anthony Birch, Elaine M Boyd, Naresha Gunin, Mike A Scott, Wendy N Erber, the Cancer Genome Project, Anthony R Green

*These authors contributed equally to this study

Members listed at end of report

Summary

Identification of an Acquired JAK2 Mutation in Polycythemia Vera

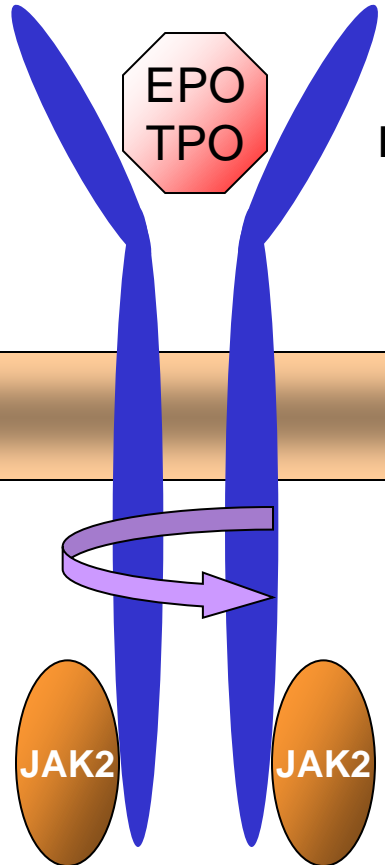
Runxiang Zhao¹, Shu Xing^{1,2}, Zhe Li^{1,2}, Xueqi Fu², Qingshan Li², Sanford B. Krantz¹, Zhizhuang Joe Zhao^{1,2*}

¹Hematology/Oncology Division, Department of Medicine, Vanderbilt-Ingram Cancer Center, Vanderbilt University, Nashville, TN 37232, USA.

²Edmond H. Fischer Signal Transduction Laboratory, College of Life Sciences, Jilin University, Changchun, 130023, China.

Familia de proteínas JAK

- Formada por 4 miembros:
 - JAK1
 - JAK2
 - JAK3
 - Tyk2 (tyrosine kinase 2)
 - Tirocinquinasas
 - Interacciona con el dominio intracitoplasmático de receptores de membrana
-



Receptor de citocinas tipo I
EPOR
TPOR
G-CSF

CITOPLASMA

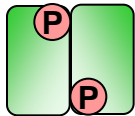
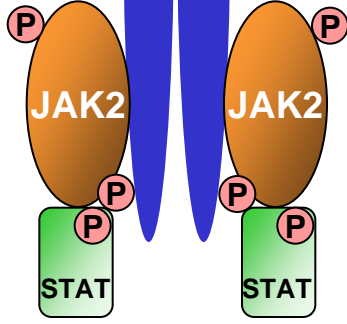
NÚCLEO



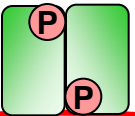


Receptor de citocinas tipo I
EPOR
TPOR
G-CSF

CITOPLASMA



NÚCLEO



Transcripción de genes

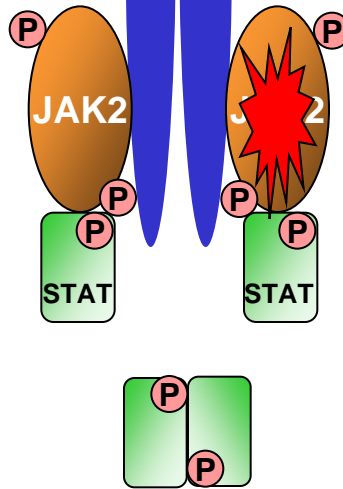




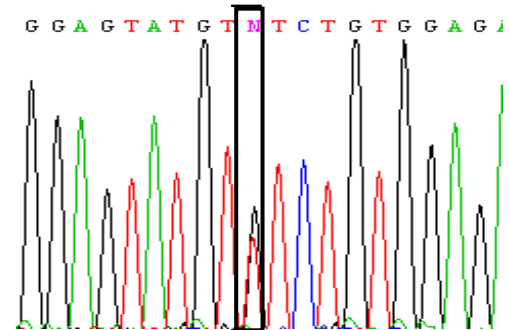
Receptor de citocinas tipo I

EPOR
TPOR
G-CSF

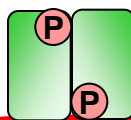
CITOPLASMA



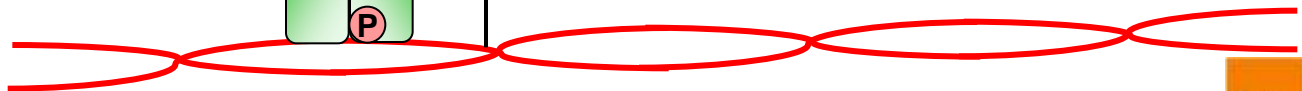
G>T
GTC → TTC
Val (V) → Phe (F)



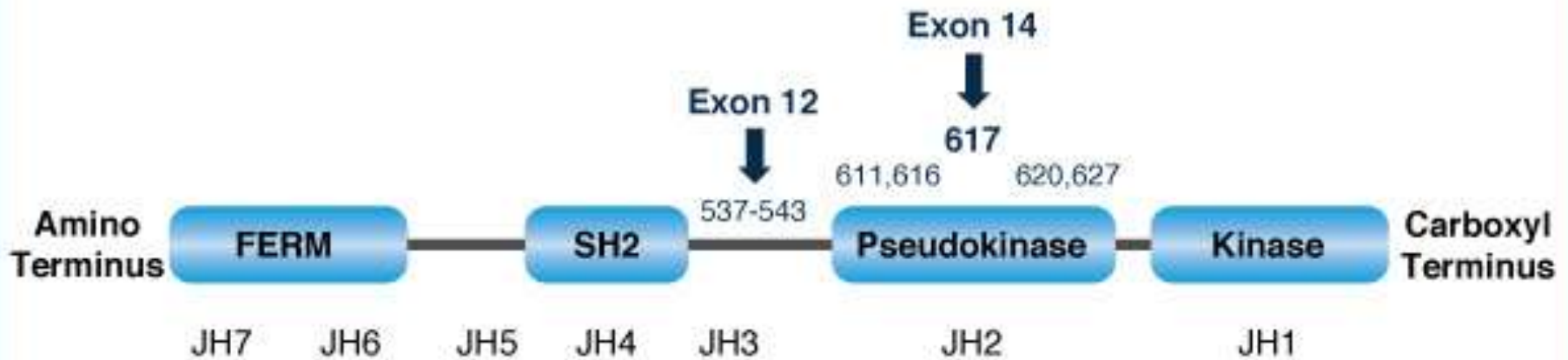
NÚCLEO



Transcripción de genes



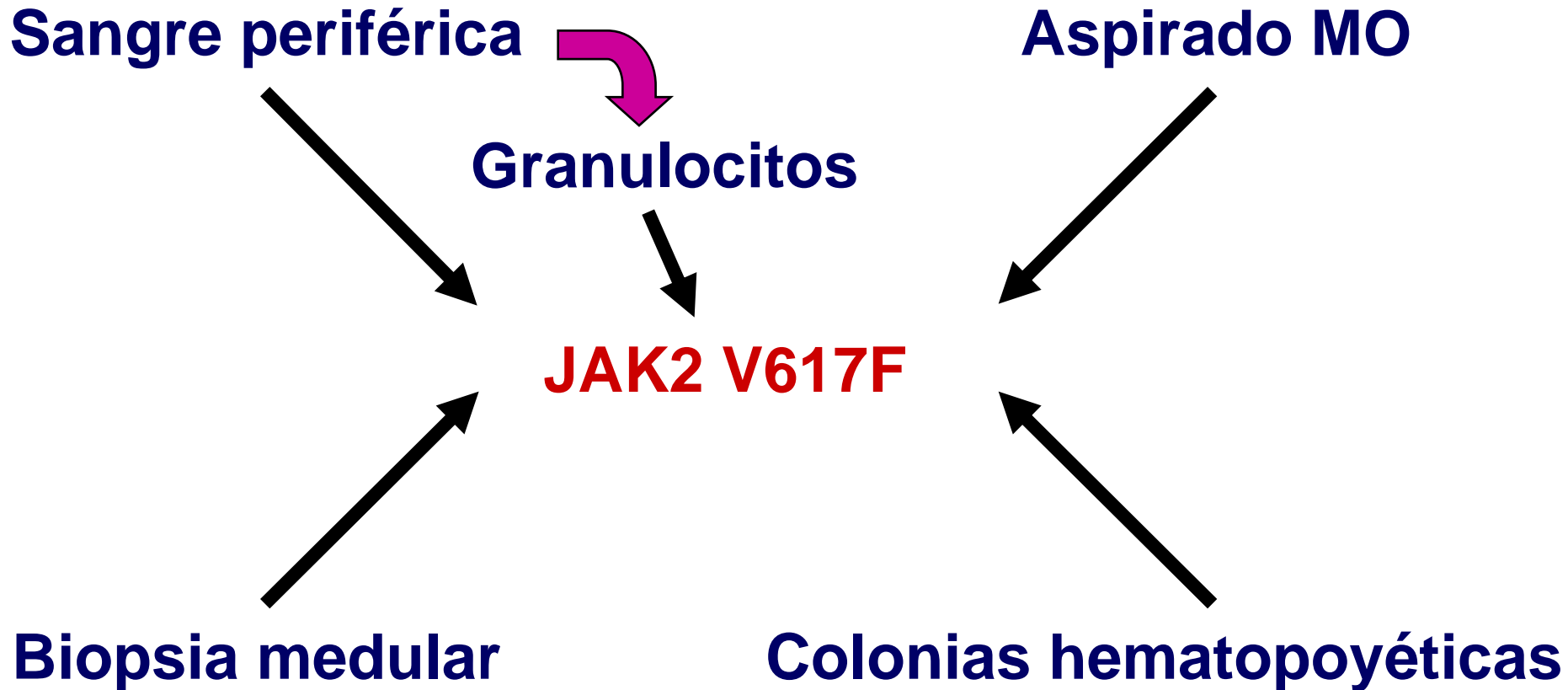
JAK2 Mutations Implicated in MPNs⁶



Frecuencia de la mutación JAK2V617F en NMs Ph-negativas

PV	95%
TE	60 %
MFP	60 %

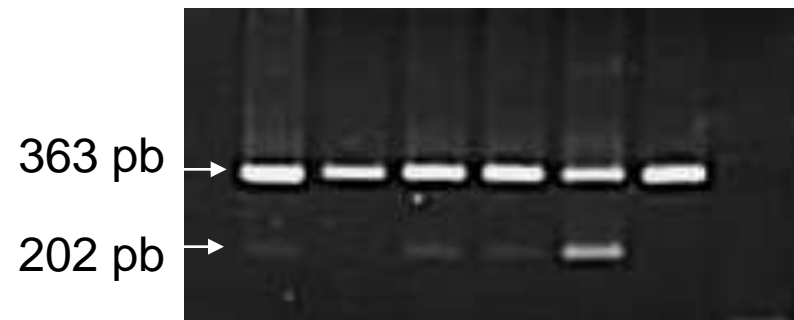
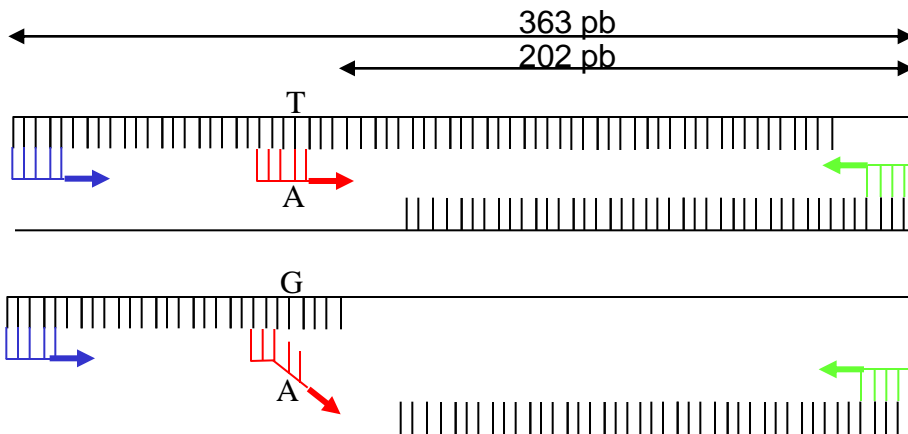
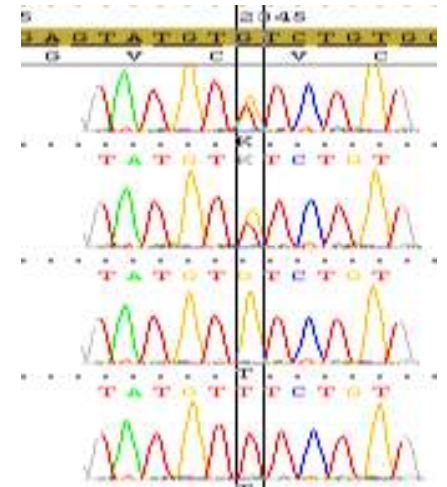
Detección de la mutación JAK2V617F



Detección de la mutación V617F JAK2

Técnicas de detección

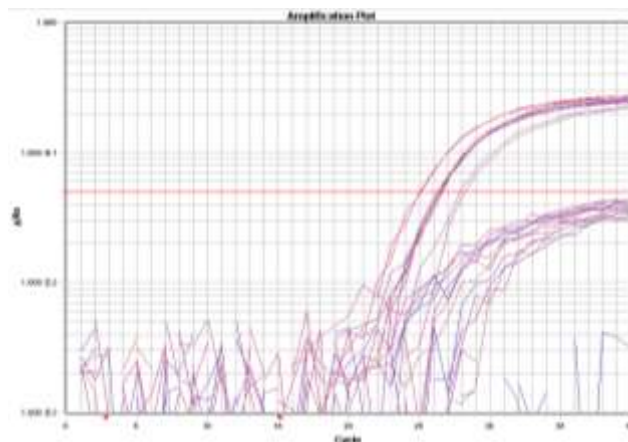
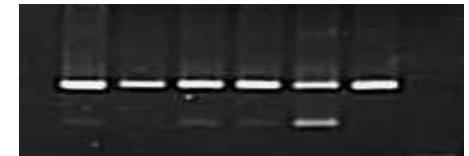
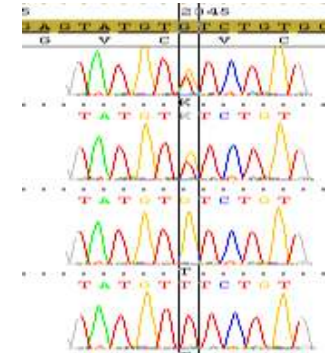
- Secuenciación directa
 - Terminadores marcados y análisis en secuenciador ABI 3100
- PCR alelo-específica (Baxter et al, 2005)
 - ADN de granulocitos
 - Análisis en gel de agarosa



Detección de la mutación V617F *JAK2*

Técnicas de detección

- Secuenciación directa
 - Terminadores marcados y análisis en secuenciador ABI 3100
- PCR alelo-específica (Baxter et al, 2005)
 - ADN de granulocitos
 - Análisis en gel de agarosa
- PCR alelo-específica en tiempo real



Mutados

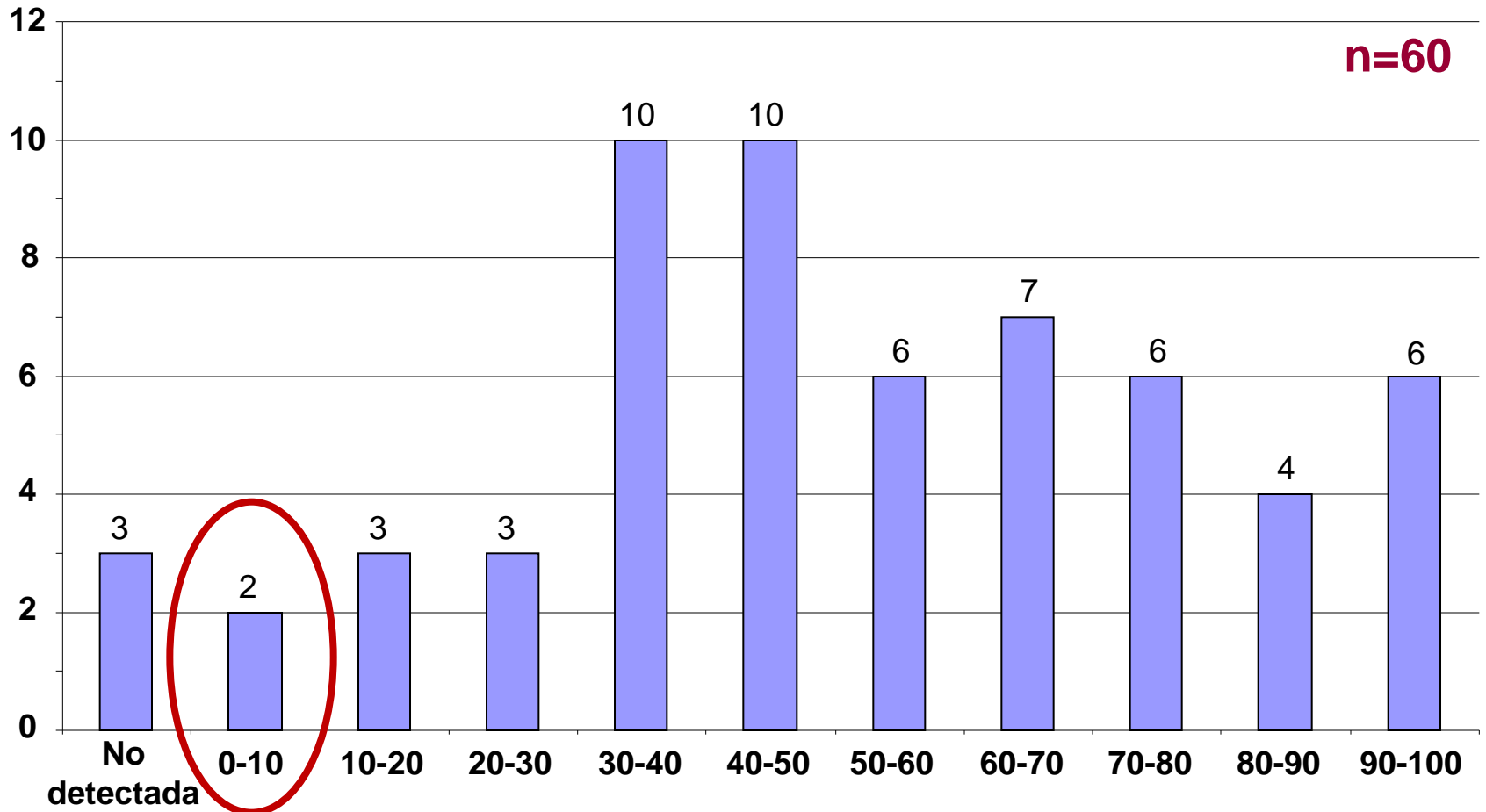
No mutados

Técnicas de detección de mutaciones

• Secuenciación directa (Sanger)	10-20%	Conocidas + Nuevas
• Pirosecuenciación	5%	Conocidas + Nuevas
• High Resolution Melting	2,5-5%	Conocidas + Nuevas
• PCR alelo-específica		
– ARMS	3%	Conocidas
– TaqMan	0,1%	Conocidas
– FRET	0,1%	Conocidas
• Técnicas de enriquecimiento de alelo mutado		
– PNA-LNA clamp	1%	Conocidas
– Digestión con enzimas de restricción	0,2%	Conocidas
– COLD-PCR	1-10%	Conocidas + Nuevas
• CAST-PCR	0,01%	Conocidas
• d-HPLC	0,1-1%	Conocidas + Nuevas
• PCR-RFLP	5-10%	Conocidas

Carga alélica de *JAK2V617F* en PV

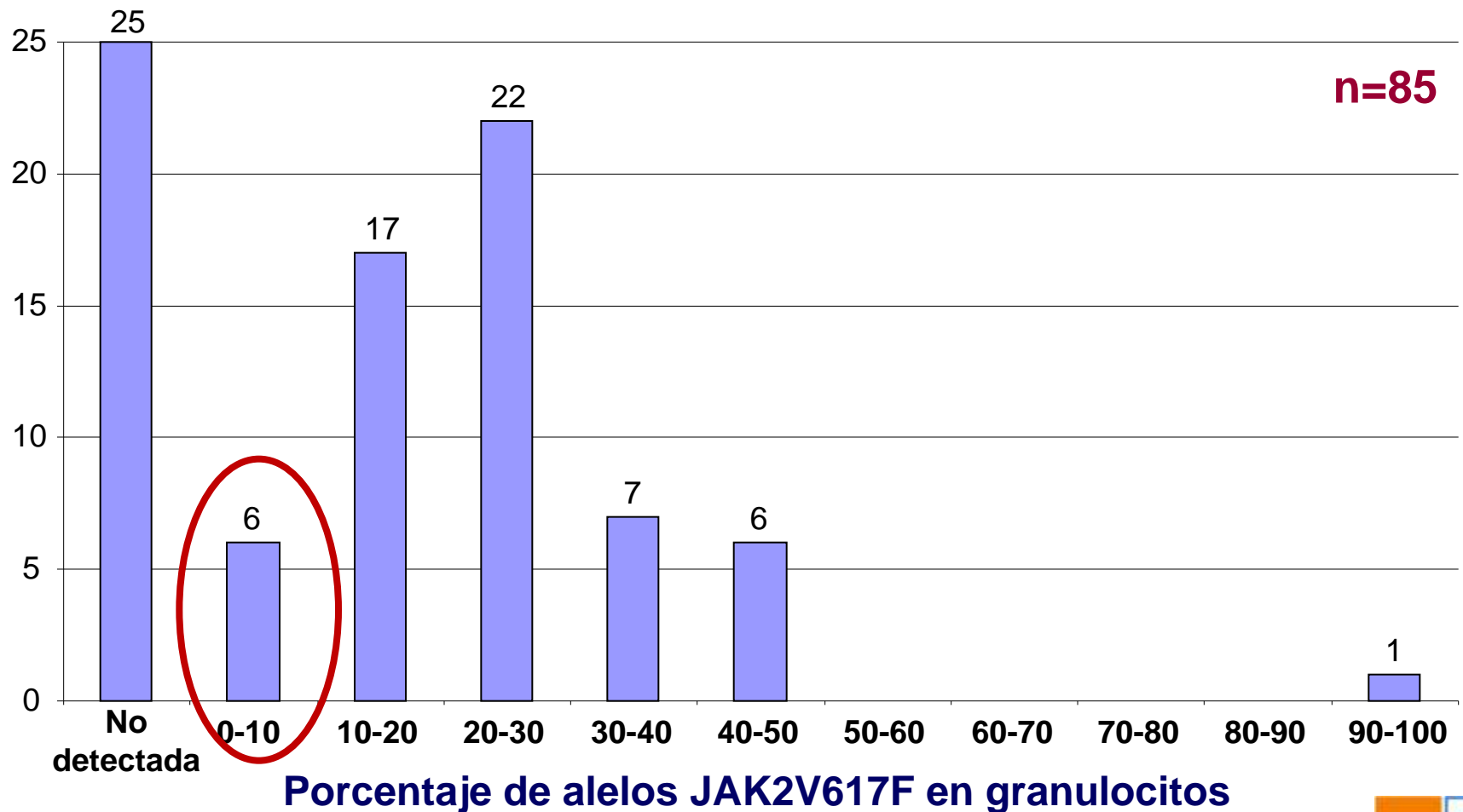
Experiencia del Hospital del Mar

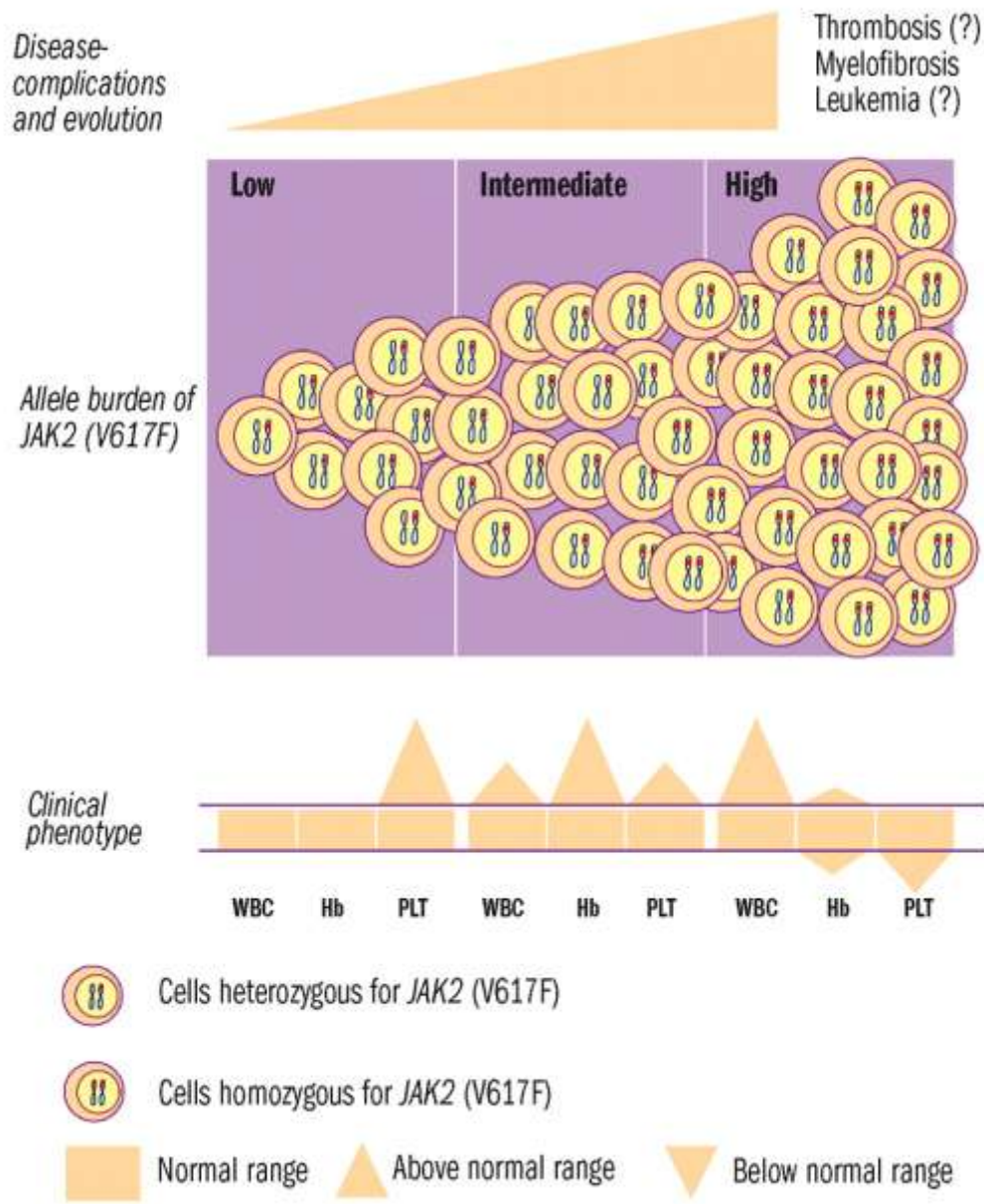


Porcentaje de alelos *JAK2V617F* en granulocitos

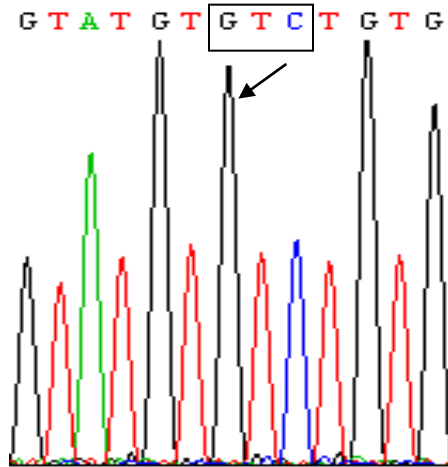
Carga alélica de *JAK2V617F* en TE

Experiencia del Hospital del Mar

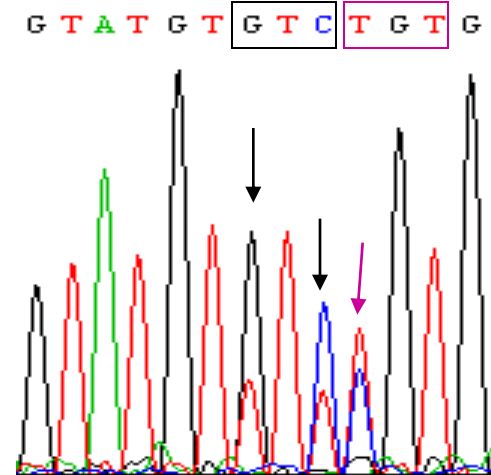




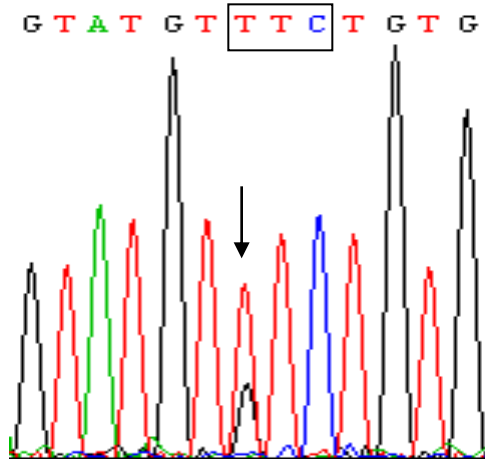
Mutaciones diferentes/adicionales a c.1849 G>T



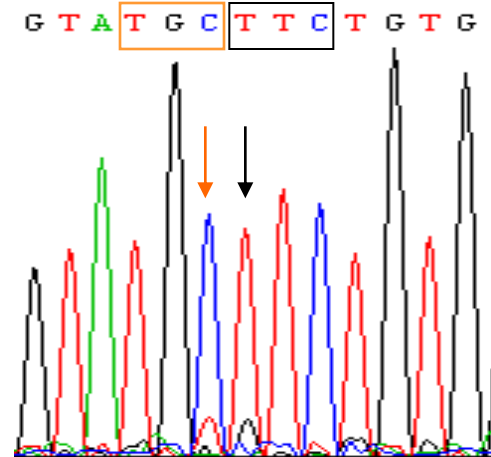
Wild type



V617F C618R



V617F

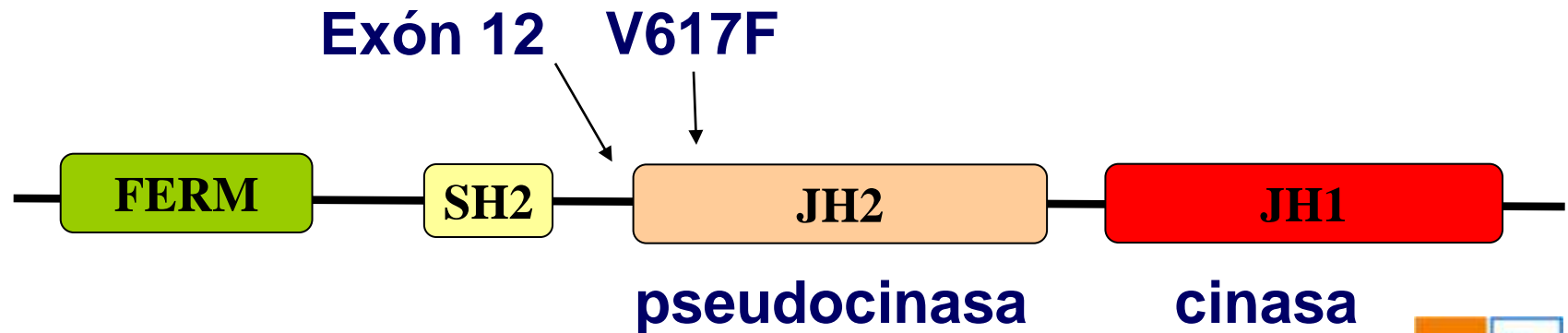


C616C V617F

ORIGINAL ARTICLE

JAK2 Exon 12 Mutations in Polycythemia Vera and Idiopathic Erythrocytosis

Linda M. Scott, Ph.D., Wei Tong, Ph.D., Ross L. Levine, M.D.,
Mike A. Scott, Ph.D., Philip A. Beer, M.R.C.P., M.R.C.Path.,
Michael R. Stratton, M.D., Ph.D., P. Andrew Futreal, Ph.D.,
Wendy N. Erber, M.D., Mary Frances McMullin, F.R.C.P., F.R.C.Path.,
Claire N. Harrison, M.R.C.P., M.R.C.Path., Alan J. Warren, F.R.C.Path., F.Med.Sci.,
D. Gary Gilliland, M.D., Ph.D., Harvey F. Lodish, Ph.D.,
and Anthony R. Green, F.R.C.Path., F.Med.Sci.



SUBSTITUTIONS

wildtype

F533IK539L

F537IK539L

H538QK539L

H538DK539LI540S

K539L

K539LL545V

I540T

D544G

L545S

F547L

F547V

(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVFHKIRNEDLIF
(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMIQMVFHLIRNEDLIF
(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVIHLIRNEDLIF
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(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVFHKIRNEGLIF
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(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVFHKIRNEDLIV

DELETIONS

wildtype

F537-K539delinsK

F537-K539del

F537-K539delinsL

H538del

H538-K539del

H538-K539delinsF

H538-K539delinsI

H538-K539delinsL

I540-N542delinsS

I540-N542delinsK

I540-E543delinsKK

I540-E543delinsMK

I540-D544delinsMK

I540S,R541-E543delinsK

R541-E543delinsK

R541-D544del

N542-E543del

N542-D544delinsN

E543-D544del

D544-L545del

(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVFHKIRNEDLIF
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DUPLICATIONS

wildtype

V536-I546dup11

V536-F547dup12

V536F, F37-I546dup10

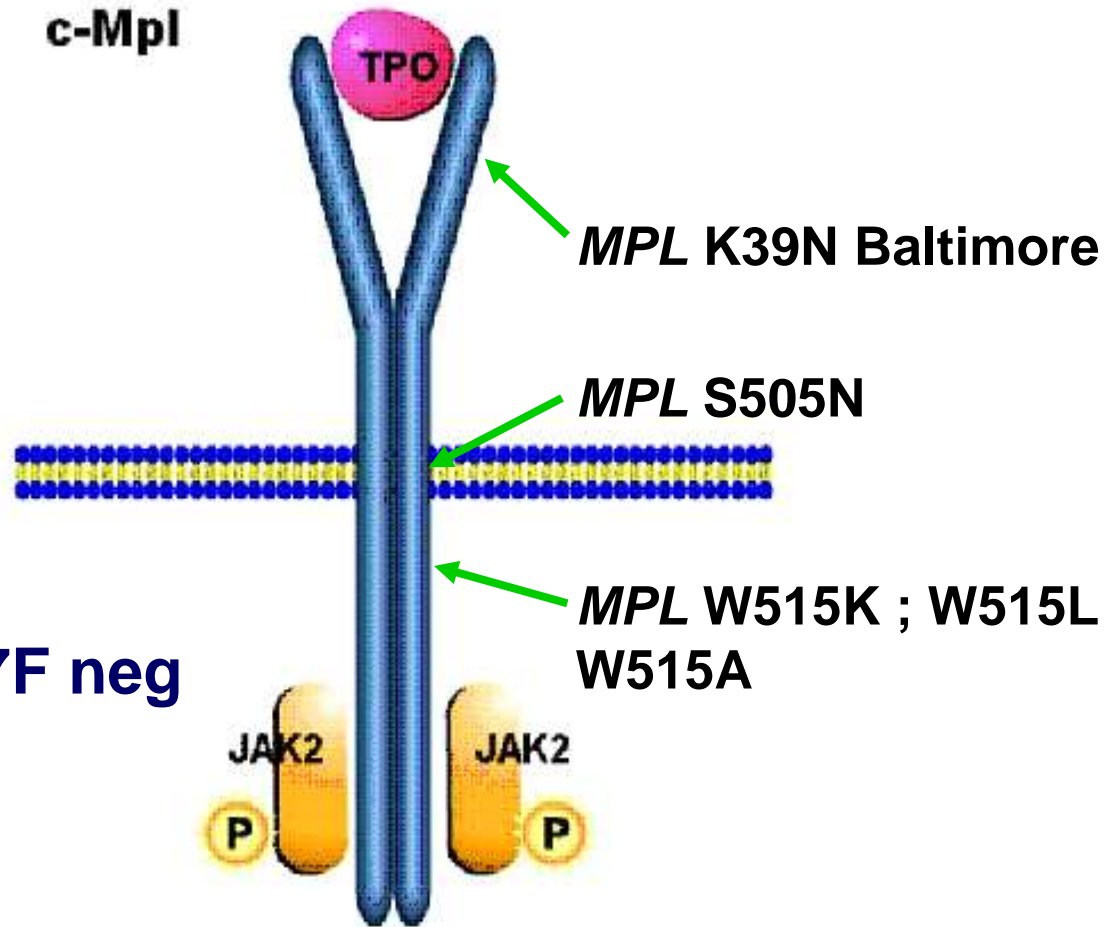
F537-F547dup11

F537-I546dup10,F547L

F547L, I540-F547dup8

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(D) KSNLLVFRTNGVSDVPTSPTLQRPTHMNQMVFHKIRNEDLILIRNEDLIF

Mutaciones descritas en el gen *MPL*



TE: 1-3%
8-15% JAK2V617F neg

MFP: 5-9%

Criteria diagnósticos de las NMs OMS 2008

CRITERIA FOR POLYCYTHEMIA VERA (PV)

Diagnosis requires the presence of both major criteria and one minor criterion or the presence of the first major criterion together with two minor criteria:

Major criteria

1. Hemoglobin > 18.5 g/dL in men, 16.5 g/dL in women or other evidence of increased red cell volume*
2. Presence of *JAK2* V617F or other functionally similar mutation such as *JAK2* exon 12 mutation

Minor criteria

1. Bone marrow biopsy showing hypercellularity for age with trilineage growth (panmyelosis) with prominent erythroid, granulocytic, and megakaryocytic proliferation
2. Serum erythropoietin level below the reference range for normal
3. Endogenous erythroid colony formation *in vitro*

CRITERIA FOR ESSENTIAL THROMBOCYTHEMIA (ET)

Diagnosis requires meeting all four criteria:

1. Sustained platelet count $\geq 450 \times 10^9/L$ ^a
2. Bone marrow biopsy specimen showing proliferation mainly of the megakaryocytic lineage with increased numbers of enlarged, mature megakaryocytes. No significant increase or left-shift of neutrophil granulopoiesis or erythropoiesis
3. Not meeting WHO criteria for polycythemia vera^b, primary myelofibrosis^c, *BCR-ABL1* positive CML^d or myelodysplastic syndrome^e or other myeloid neoplasm
4. Demonstration of *JAK2* V617F or other clonal marker, or in the absence of *JAK2* V617F, no evidence of reactive thrombocytosis^f

CRITERIA FOR PRIMARY MYELOFIBROSIS (PMF)

Diagnosis requires meeting all three major criteria and two minor criteria:

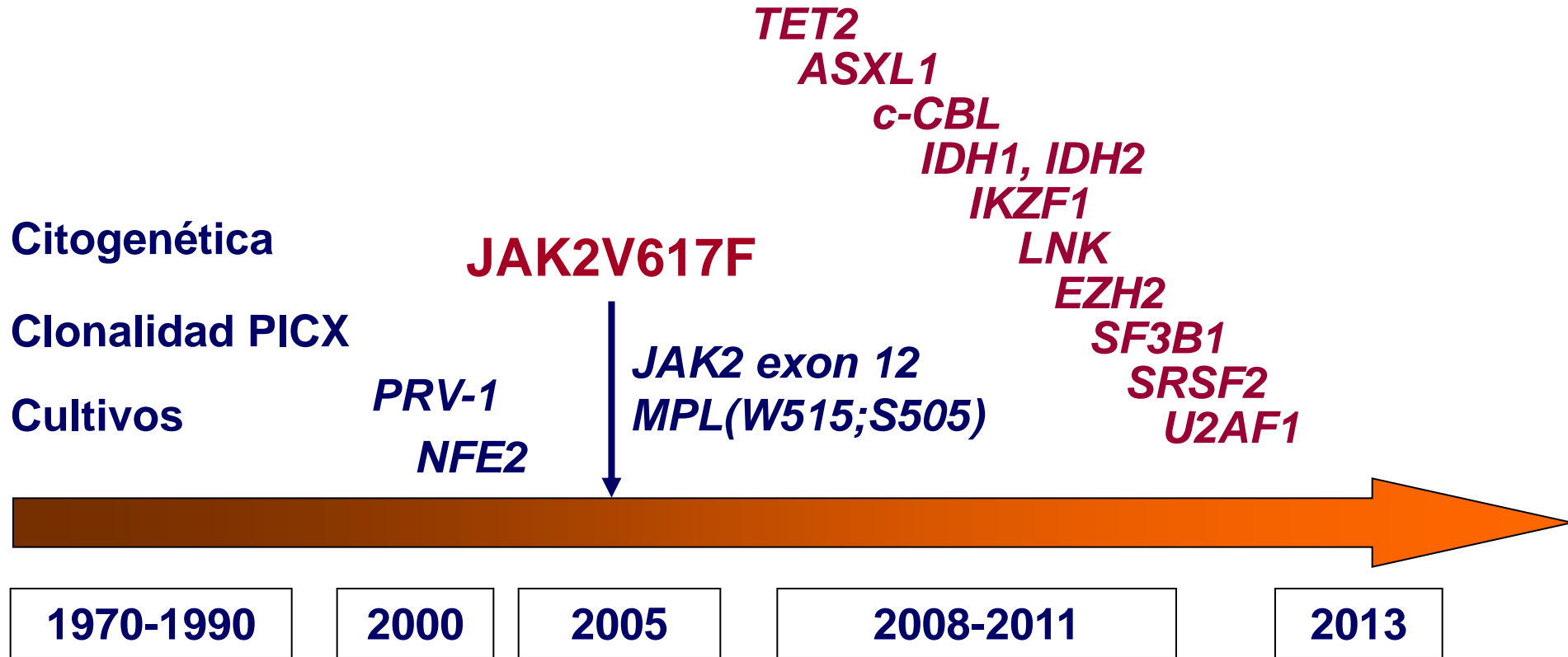
Major criteria

1. Presence of megakaryocyte proliferation and atypia,^a usually accompanied by either reticulin and/or collagen fibrosis,
or,
in the absence of significant reticulin fibrosis, the megakaryocyte changes must be accompanied by an increased bone marrow cellularity characterized by granulocytic proliferation and often decreased erythropoiesis (i.e., pre-fibrotic cellular-phase disease)
2. Not meeting WHO criteria for polycythemia vera,^b *BCR-ABL1*+ chronic myelogenous leukemia,^c myelodysplastic syndrome,^d or other myeloid disorders
3. Demonstration of *JAK2* V617F or other clonal marker (e.g. *MPLW515K/L*),
or
in the absence of the above clonal markers, no evidence that bone marrow fibrosis is secondary to infection, autoimmune disorder or other chronic inflammatory condition, hairy cell leukemia or other lymphoid neoplasm, metastatic malignancy, or toxic (chronic) myelopathies^e

Minor criteria

1. Leukoerythroblastosis^f
2. Increase in serum lactate dehydrogenase level^f
3. Anemia^f
4. Palpable splenomegaly^f

Marcadores moleculares en NMPs



Otros genes implicados en NM

Señalización

LNK
CBL

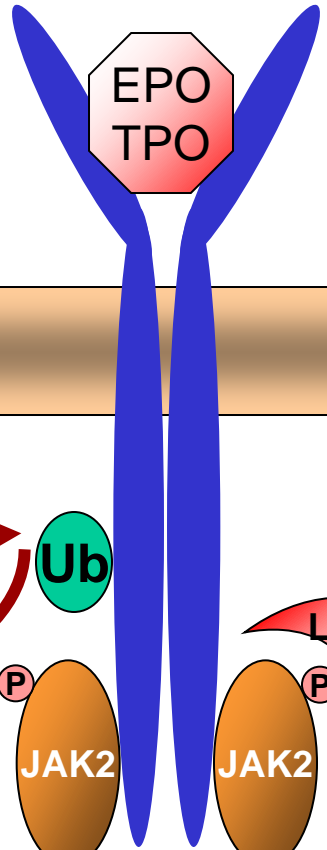
Epigenética

Metilación ADN
TET2
IDH1/2
DNMT3A

Remodelación
de la cromatina
ASXL1
EZH2

Splicing ARNm

SF3B1
SRSF2
U2AF1



Receptor de citocinas tipo I
 EPOR
 TPOR
 G-CSF

CITOPLASMA

Mutaciones *LNK* :

PV	Infrecuente
TE	3 - 6 %
MFP	3 - 6%

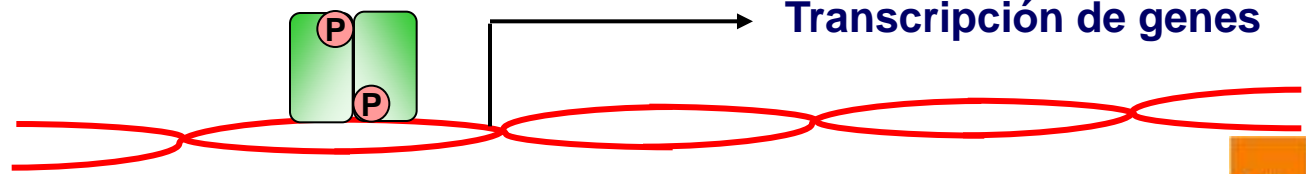
Internalización
 y degradación

Mutaciones *CBL* :

PV	Infrecuente
TE	Infrecuente
MFP	5 - 10 %

NÚCLEO

Transcripción de genes



Otros genes implicados en NM

Señalización

LNK
CBL

Epigenética

Metilación ADN

TET2
IDH1/2
DNMT3A

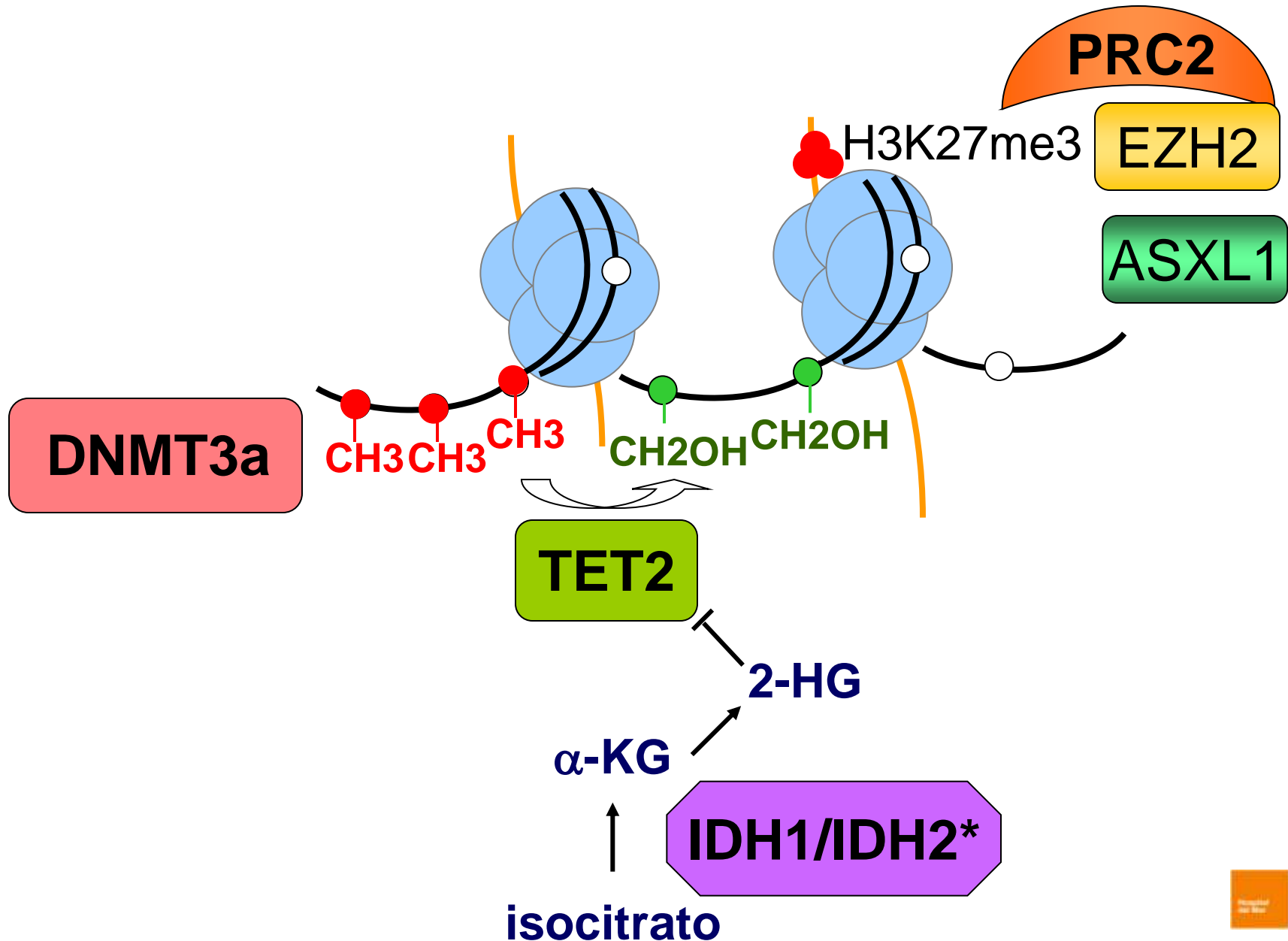
Remodelación
de la cromatina

ASXL1
EZH2

Splicing ARNm

SF3B1
SRSF2
U2AF1

Mutaciones Epigenéticas en NM



Otros genes implicados en NM

Señalización

LNK
CBL

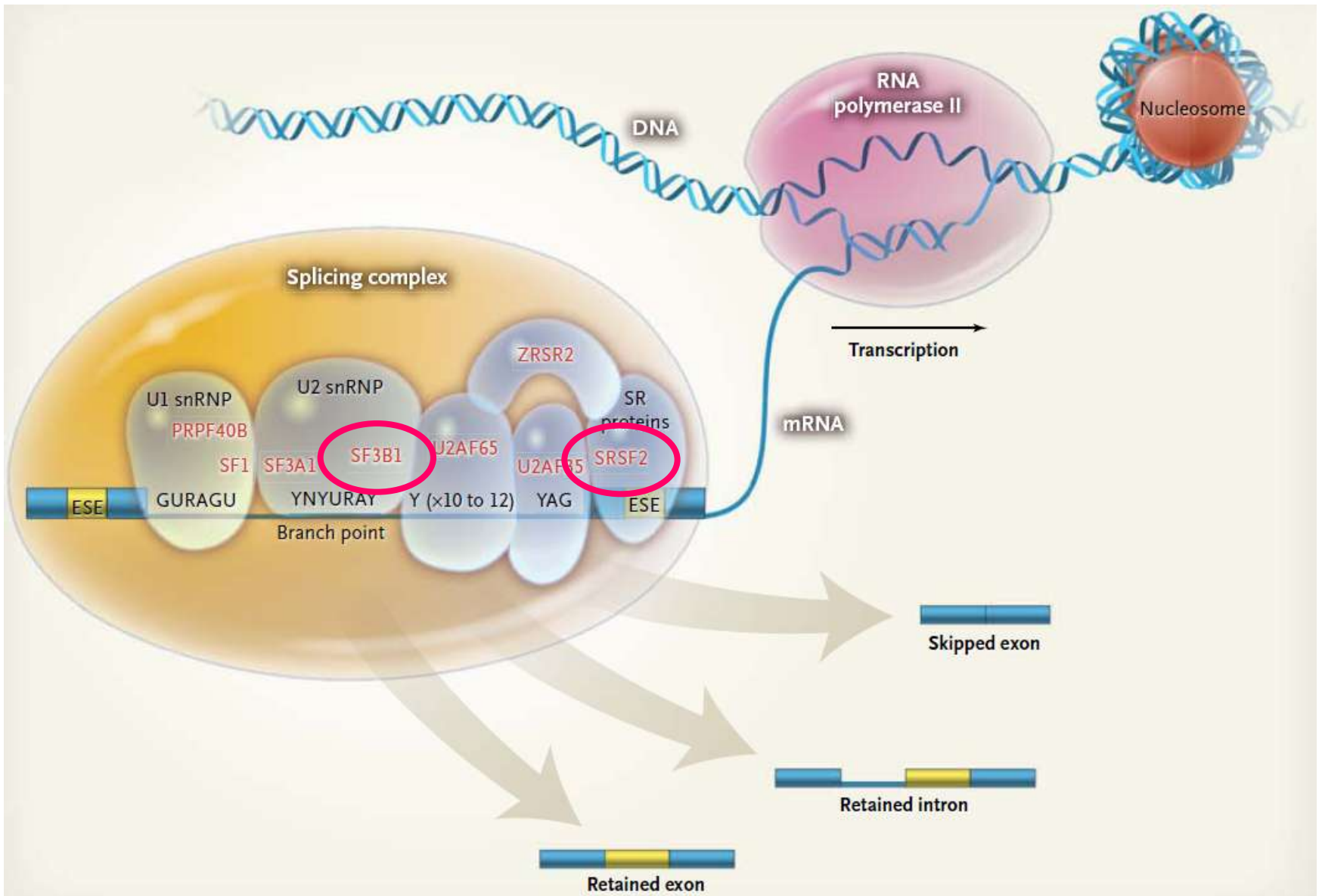
Epigenética

Metilación ADN
TET2
IDH1/2
DNMT3A

Remodelación
de la cromatina
ASXL1
EZH2

Splicing ARNm

SF3B1
SRSF2
U2AF1



Gen	PV	TE	MF
JAK2V617F	95-97	60	60
JAK2 exón12	1-2	0	0
MPL	0	3-5	5-10
CALR	Infrecuente	30	23-35
LNK/SH2B3	1	3-6	3-6
CBL	Infrecuente	Infrecuente	5-10
TET2	10-20	5	10-20
IDH1/IDH2	Infrecuente	Infrecuente	6
DNMT3A	5-10	1-5	5-10
IKZF1	Infrecuente	Infrecuente	Infrecuente
ASXL1	2-5	2-5	13-20
EZH2	1-3	1	5-10
SF3B1	Infrecuente	3	4-7
SRSF2	Infrecuente	Infrecuente	17

MF Post-PV

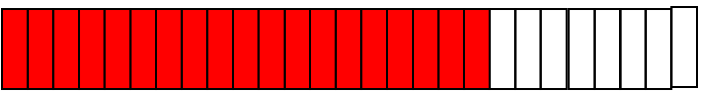
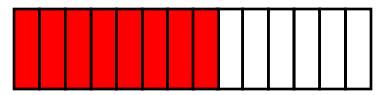
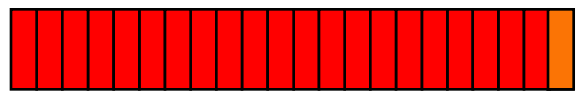
MF Post-ET

MFP

* #

*

JAK2



MPL



CALR



SF3B1



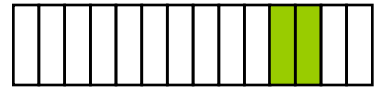
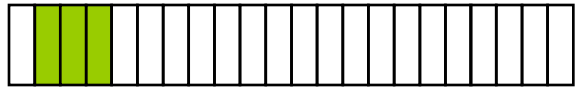
SRSF2



U2AF1



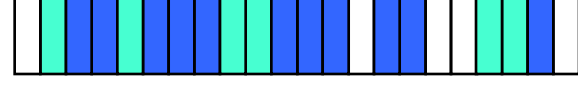
TET2



ASXL1

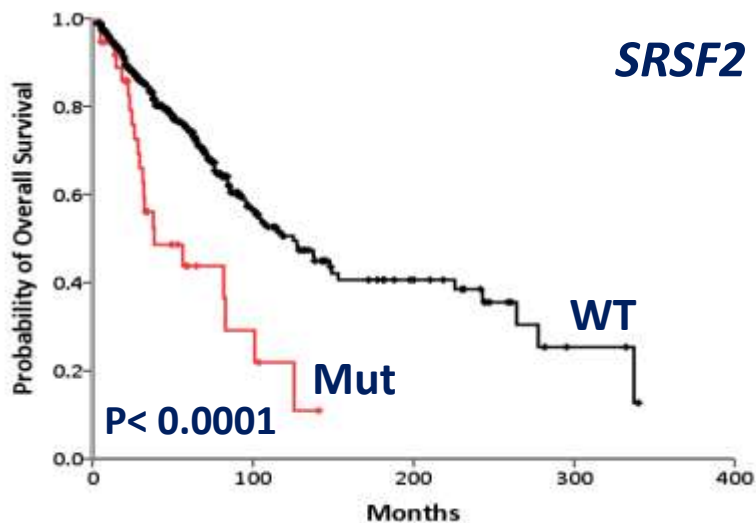
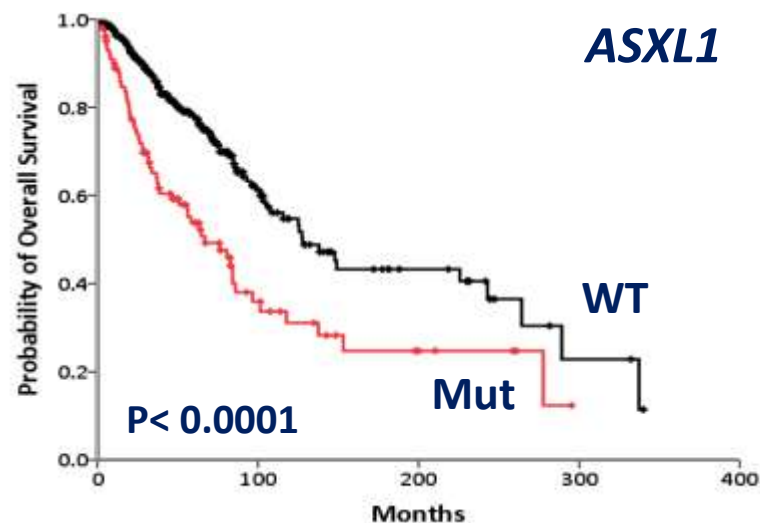
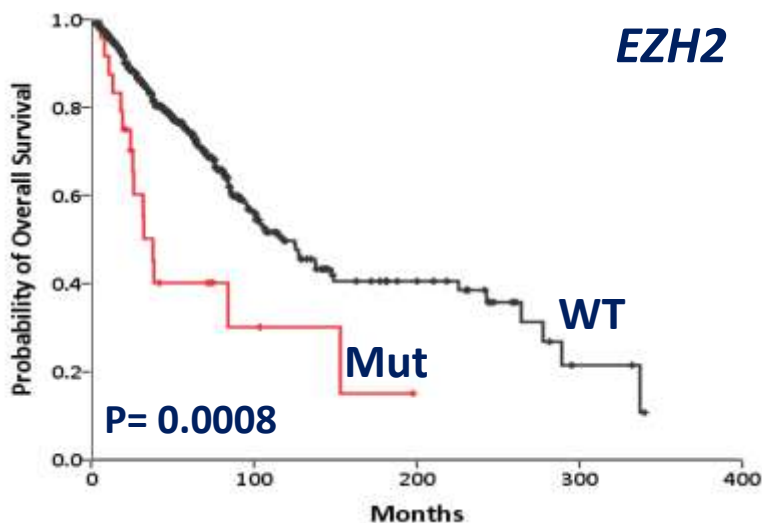


46/1



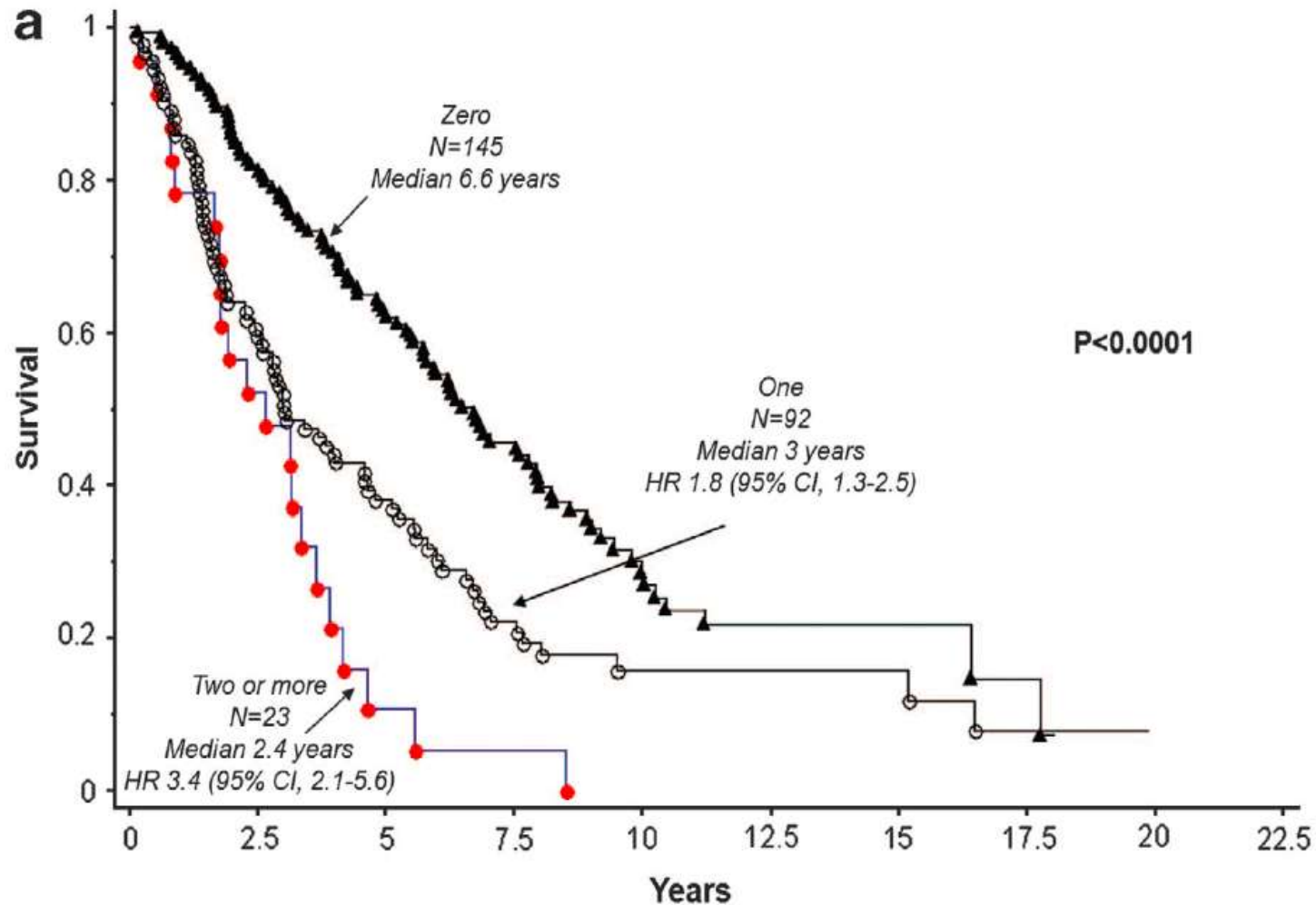
- Homocigoto
- Heterocigoto
- No haplotipo *JAK2* 46/1

Mutaciones asociadas con supervivencia global inferior en MFP– análisis multivariado

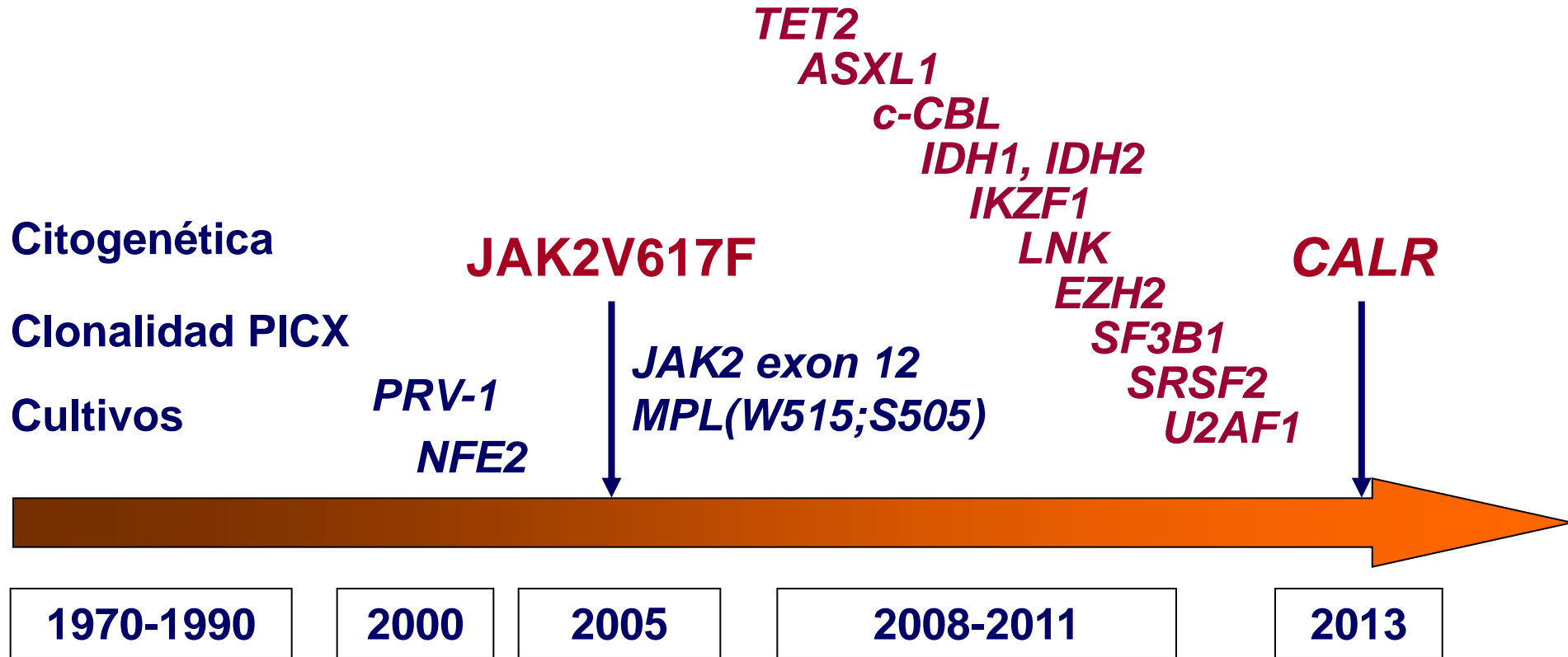


	Hazard Risk (95% CI range)	P
EZH2	1.91 (1.08-3.36)	0.025
ASXL1	2.21 (1.57-3.11)	<0.0001
SRSF2	2.60 (1.63-41.6)	<0.0001

Valor pronóstico del número de genes mutados en MFP (*ASXL1*, *SRSF2*, *EZH2* e *IDH*)



Marcadores moleculares en NMPs



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 Milanese, 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.

Secuenciación de exoma

6 MFP

Secuenciación de exoma

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

151 NMPs:

48 PV

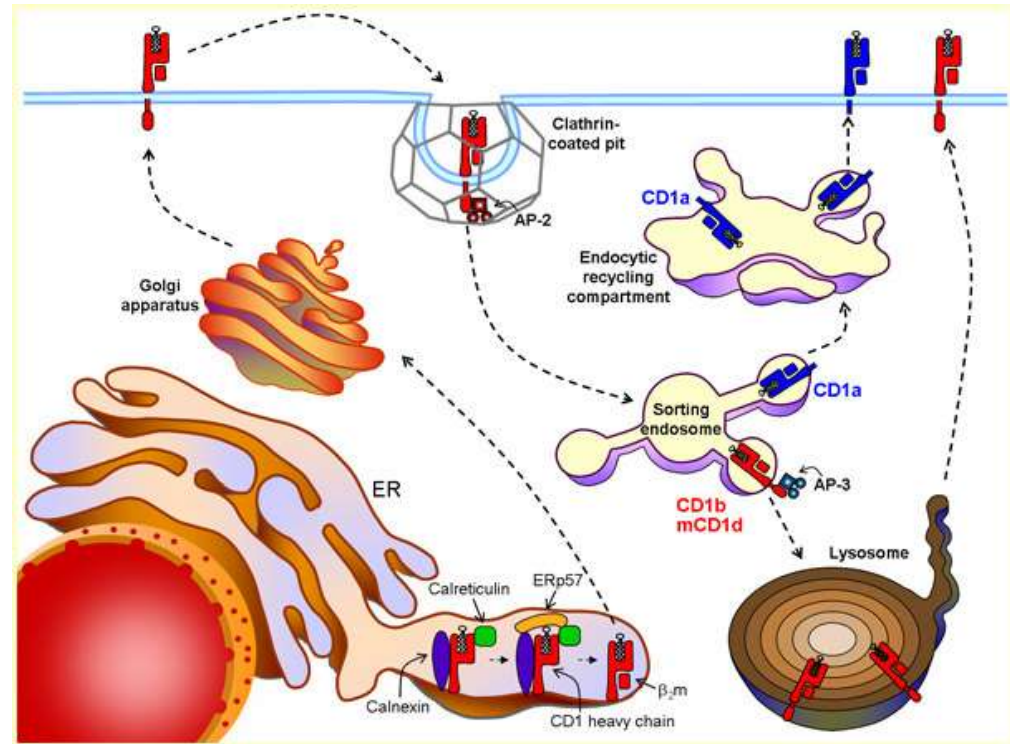
62 TE

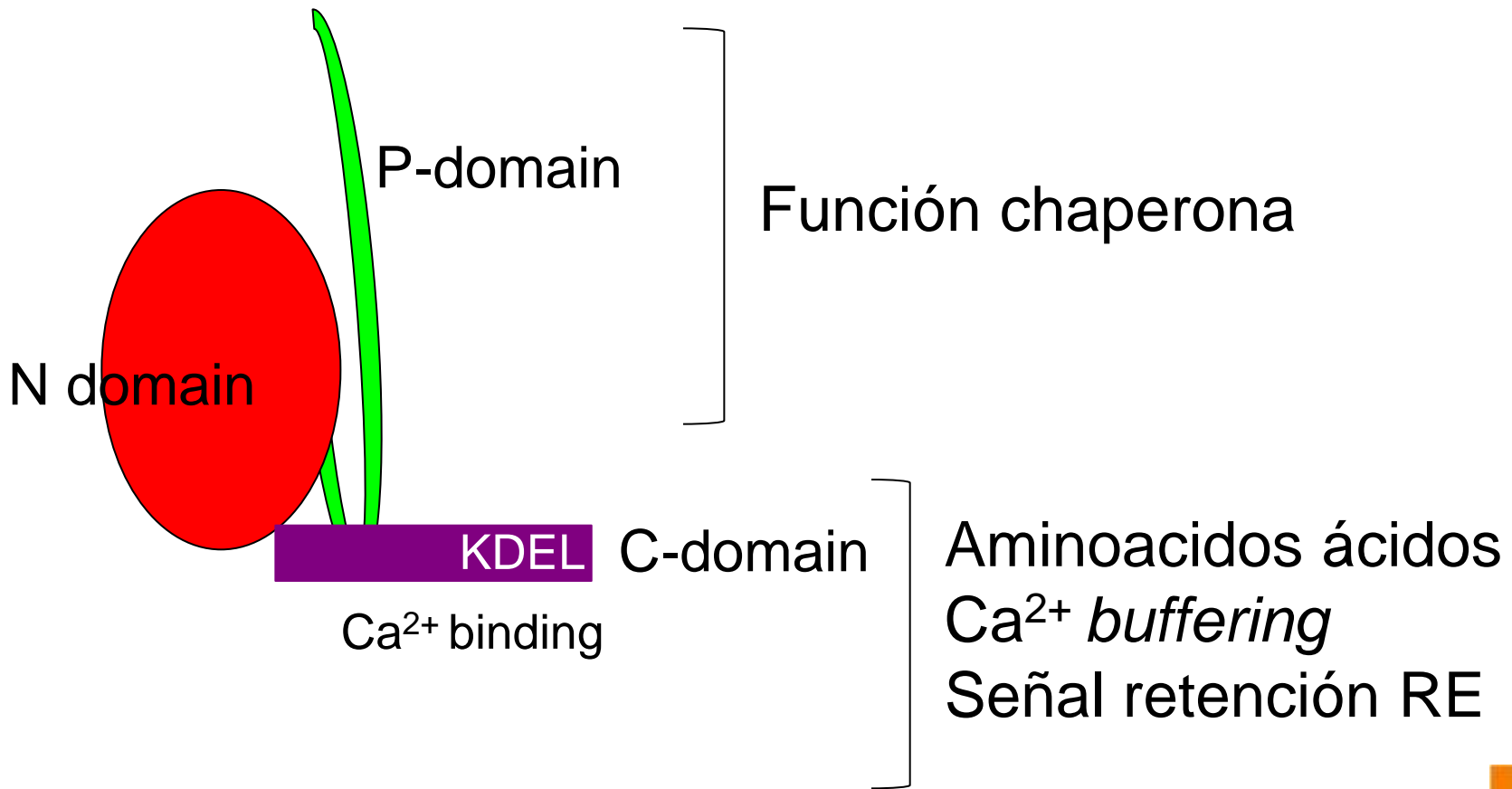
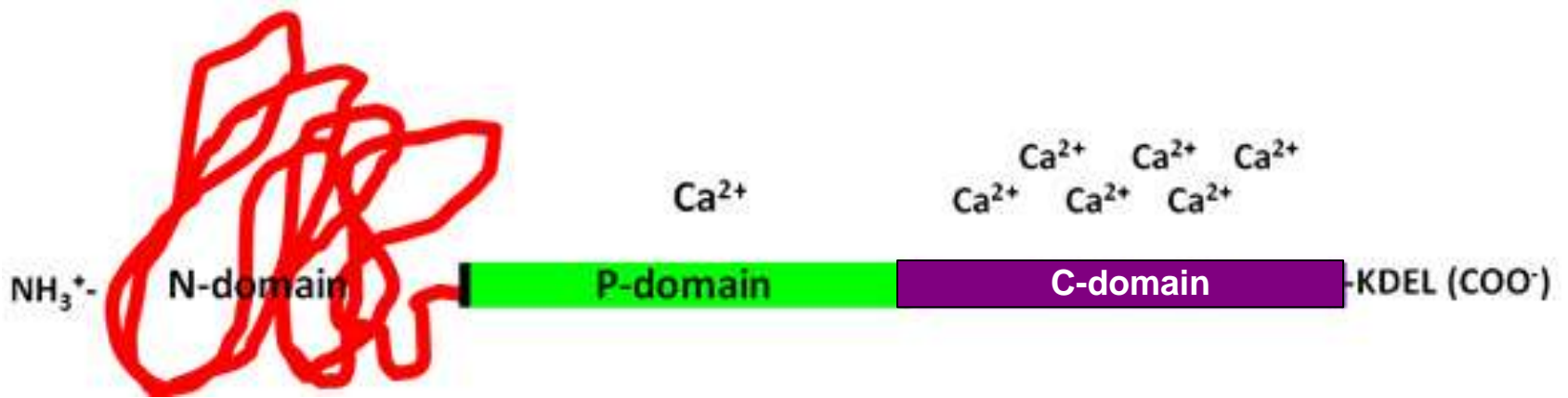
39 MF

2 uMPN

Calreticulina

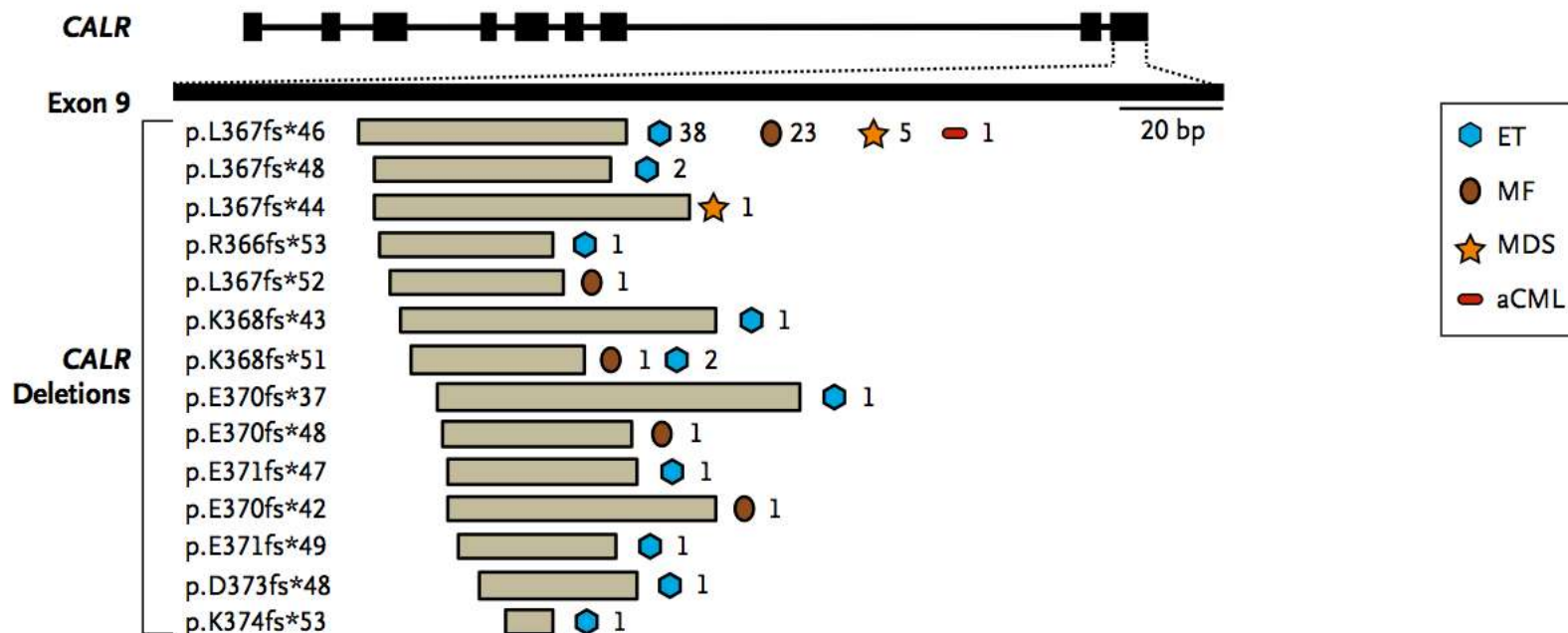
- Chaperona codificada por gen *CALR*
- Localización en retículo endoplasmático (RE):
 - Plegamiento de las glicoproteínas
 - Homeostasis Ca^{2+}
- Fuera del RE:
 - Regulación de proliferación, apoptosis, fagocitosis y respuesta inmune



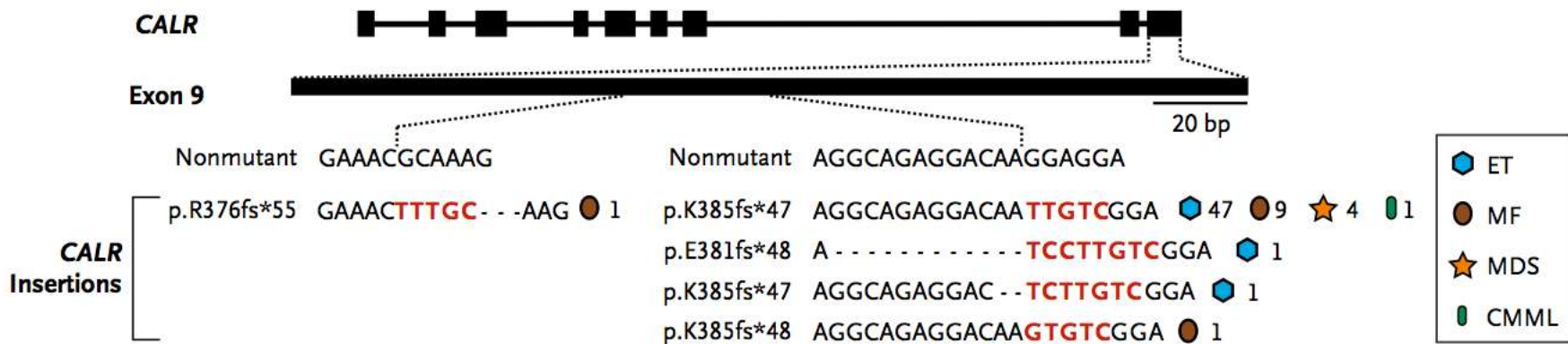


Mutaciones en CALR

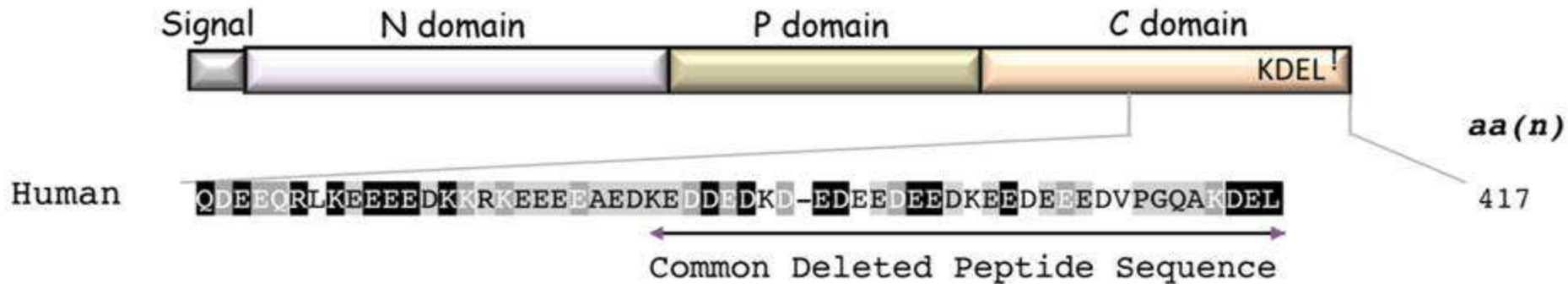
Genomic Location of CALR Deletions



Genomic Location of CALR Insertions



Las mutaciones en *CALR* originan un nuevo extremo C terminal



Deletions

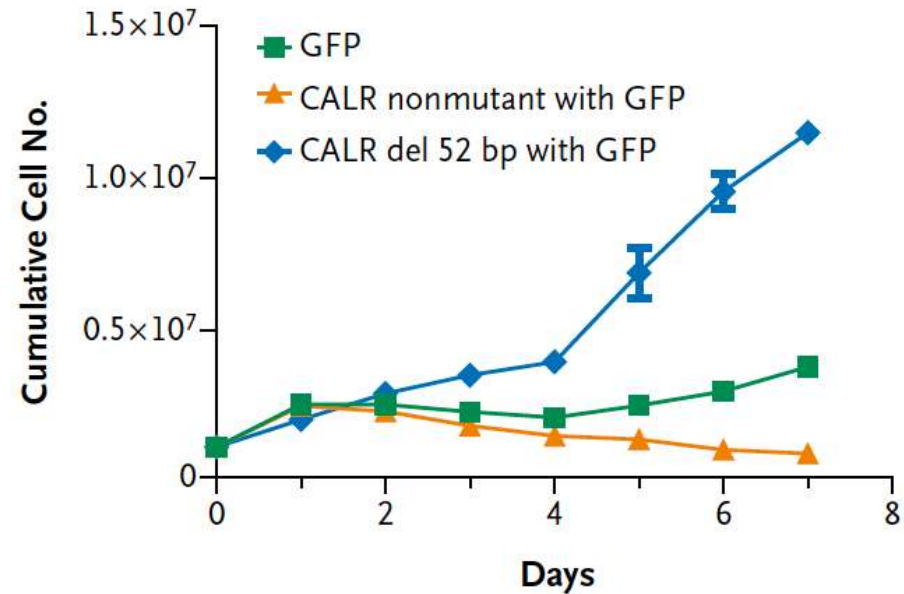
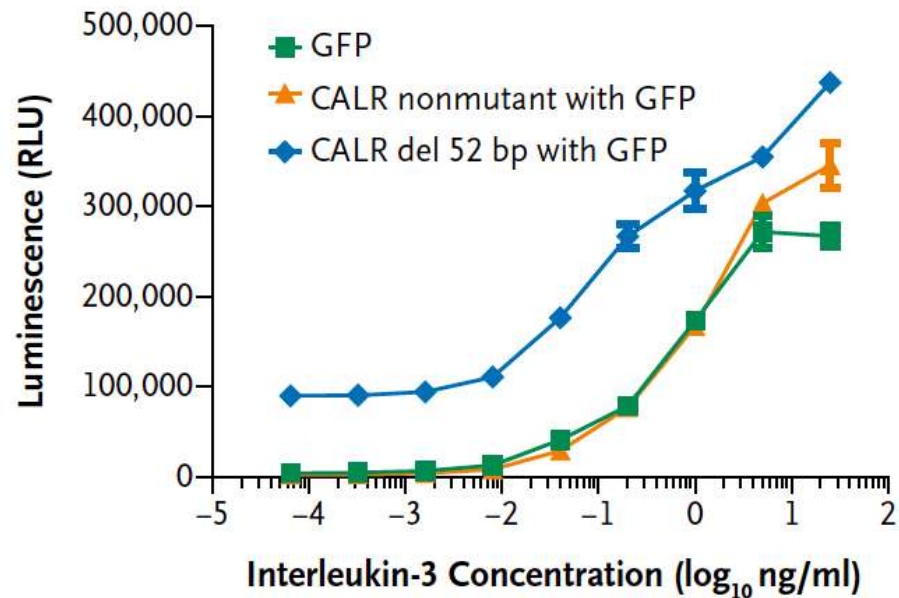
L367fs*46	QDEE -----	<u>QRTTRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLOGWTEA</u>	411
E370fs*43	QDEEQRLKE -----	<u>VMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLOGWTEA</u>	411
E370fs*48	QDEEQRLKE -----	<u>QRTTRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLOGWTEA</u>	416

Insertions

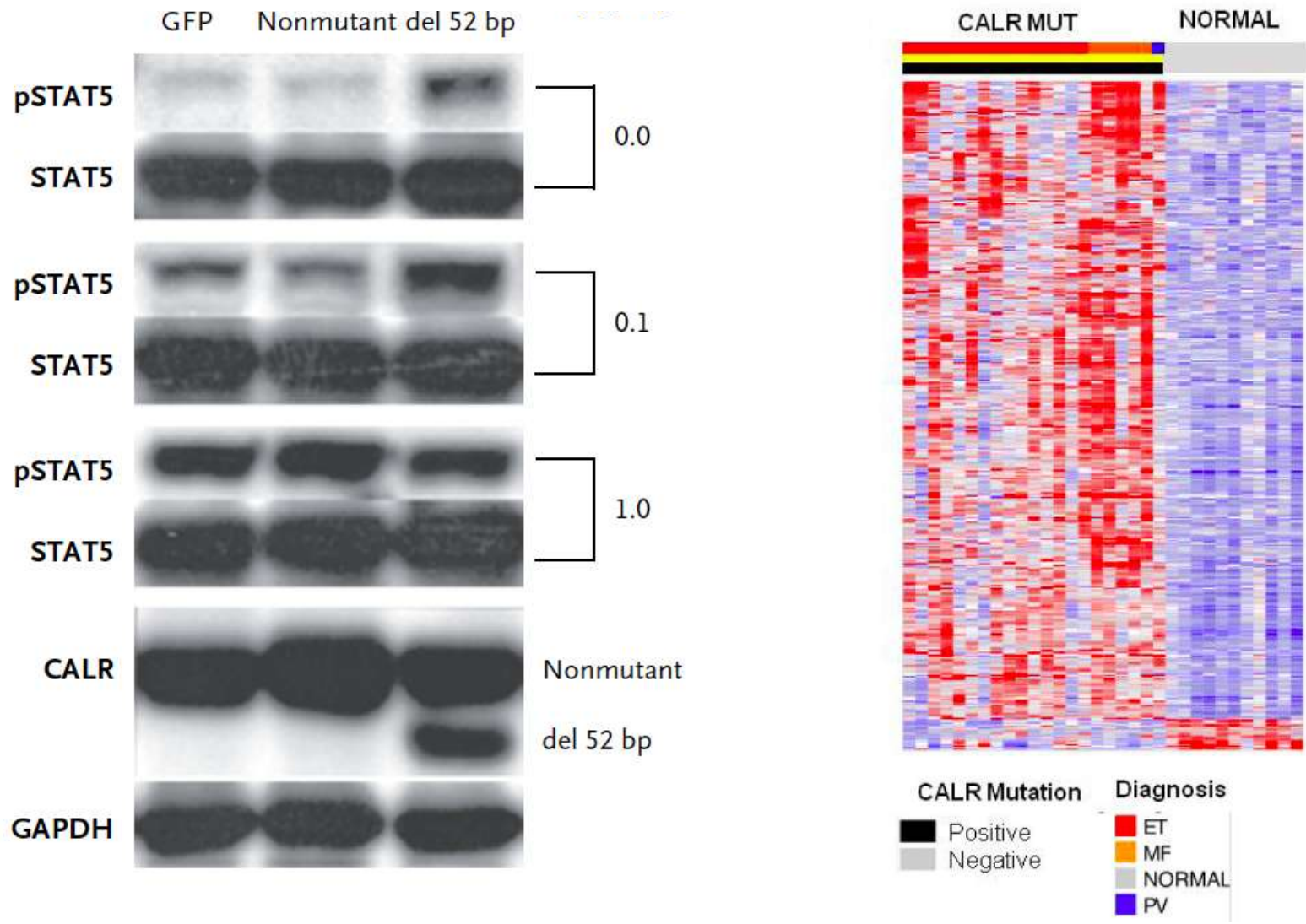
K385fs*47	QDEEQRLKEE EEDKRRKEEE EAED	NC ▼ <u>RRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLOGWTEA</u>	430
K385fs*47	QDEEQRLKEE EEDKRRKEEE EAED	LC ▼ <u>RRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLOGWTEA</u>	430

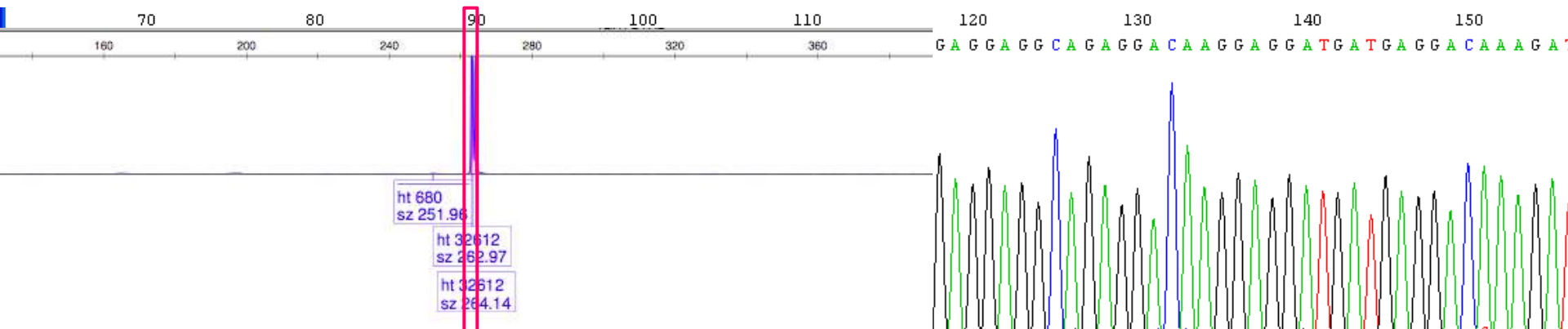
Wildtype reference sequence		AAEKQMKDKQDEEQRLKEEEEDKKRKEEEEAEDEKEDDEDKDEDEEDEDKEEDEFEEEDVPGQAKDEL-
CALR mutation	Nucleotide	Aminoacid* Change
Type 1	c.1092_1143del	AAEKQMKDKQDEEQRTRRMMRTKMRMRMRRTRRKMRRKMSPARPTSCREACLQGWTEA-
Type 2	c.1154_1155insTTGTC	AAEKQMKDKQDEEQRLKEEEEDKKRKEEEEAE ^N DCRRMMRTKMRMRMRRTRRKMRRKMSPARPTSCREACLQGWTEA-
Type 3	c.1095_1140del	AAEKQMKDKQDEEQRTRRMMRTKMRMRMRRTRRKMRRKMSPARPTSCREACLQGWTEA-
Type 4	c.1102_1135del	AAEKQMKDKQDEEQRLRRRQRTRRMMRTKMRMRMRRTRRKMRRKMSPARPTSCREACLQGWTEA-
Type 5	c.1091_1142del	AAEKQMKDKQDEGQRTRRMMRTKMRMRMRRTRRKMRRKMSPARPTSCREACLQGWTEA-
Type 6	c.1094_1139del	AAEKQMKDKQDEERRQRTRRMMRTKMRMRMRRTRRKMRRKMSPARPTSCREACLQGWTEA-
Type 8	c.1104_1137del	AAEKQMKDKQDEEQRLKRRQRTRRMMRTKMRMRMRRTRRKMRRKMSPARPTSCREACLQGWTEA-
Type 9	c.1140del	AAEKQMKDKQDEEQRLKEEEEDKKRKEEERQRTRRMMRTKMRMRMRRTRRKMRRKMSPARPTSCREACLQGWTEA-
Type 10	c.1154delinsTGTGTC	AAEKQMKDKQDEEQRLKEEEEDKKRKEEEEAE ^D MCRRMMRTKMRMRMRRTRRKMRRKMSPARPTSCREACLQGWTEA-
Type 13	c.1100_1134delinsA	AAEKQMKDKQDEEQRRRQRTRRMMRTKMRMRMRRTRRKMRRKMSPARPTSCREACLQGWTEA-
Type 16	c.1102_1137delinsCA	AAEKQMKDKQDEEQRLQRRQRTRRMMRTKMRMRMRRTRRKMRRKMSPARPTSCREACLQGWTEA-
Type 18	c.1104_1155del	AAEKQMKDKQDEEQRLKRRMMRTKMRMRMRRTRRKMRRKMSPARPTSCREACLQGWTEA-
Type 20	c.1118_1136del	AAEKQMKDKQDEEQRLKEEEE ^G RRQRTRRMMRTKMRMRMRRTRRKMRRKMSPARPTSCREACLQGWTEA-
Type 21	c.1118_1145delinsCGTTTA	AAEKQMKDKQDEEQRLKEEEEALRGQGG-
Type 22	c.1120_1123del	AAEKQMKDKQDEEQRLKEEEEDNAKRRRRQRTRRMMRTKMRMRMRRTRRKMRRKMSPARPTSCREACLQGWTEA-
Type 28	c.1131_1152del	AAEKQMKDKQDEEQRLKEEEEDKKRKRMMRTKMRMRMRRTRRKMRRKMSPARPTSCREACLQGWTEA-
Type 32	c.1153_1154delinsTGTC	AAEKQMKDKQDEEQRLKEEEEDKKRKEEEEAE ^D CRRMMRTKMRMRMRRTRRKMRRKMSPARPTSCREACLQGWTEA-
Type 34	c.1154_delinsCTTGTC	AAEKQMKDKQDEEQRLKEEEEDKKRKEEEEAE ^D T ^C RRMMRTKMRMRMRRTRRKMRRKMSPARPTSCREACLQGWTEA-
Type 36	c.1155_1156insTGTCG	AAEKQMKDKQDEEQRLKEEEEDKKRKEEEEAE ^D K ^C RRMMRTKMRMRMRRTRRKMRRKMSPARPTSCREACLQGWTEA-
Type 37 [§]	c.1091-1124del	AAEKQMKDKQDEDAKRRRRQRTRRMMRTKMRMRMRRTRRKMRRKMSPARPTSCREACLQGWTEA-
Type 38 [§]	c.1101-1152del	AAEKQMKDKQDEEQRLRRMMRTKMRMRMRRTRRKMRRKMSPARPTSCREACLQGWTEA-
Type 39 [§]	c.1109-1145del	AAEKQMKDKQDEEQRLKEE ^T RRMMRTKMRMRMRRTRRKMRRKMSPARPTSCREACLQGWTEA-
Type 40 [§]	c.1113A>C;1122-1139del	AAEKQMKDKQDEEQRLKEEDEDK ^R QRTRRMMRTKMRMRMRRTRRKMRRKMSPARPTSCREACLQGWTEA-

Las mutaciones en *CALR* originan una independencia de factores de crecimiento

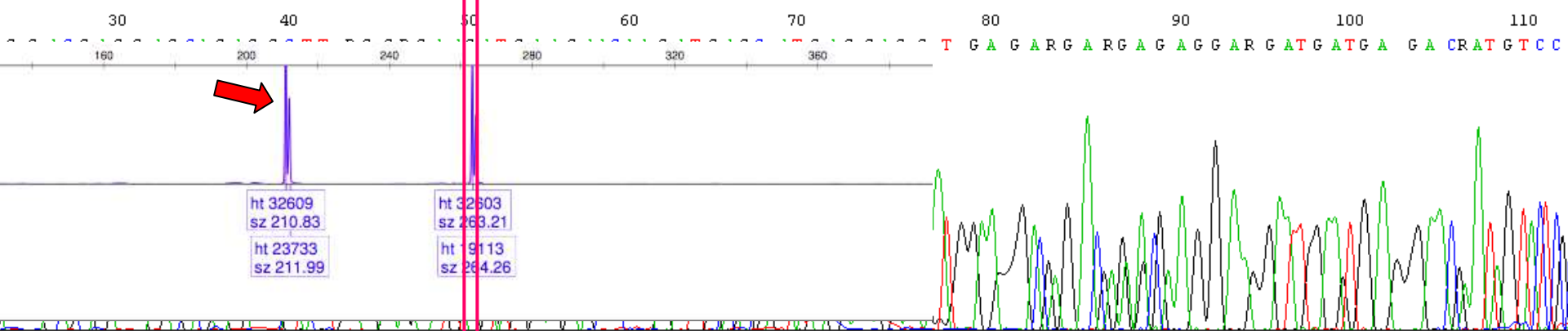


Las mutaciones en *CALR* activan la vía JAK-STAT

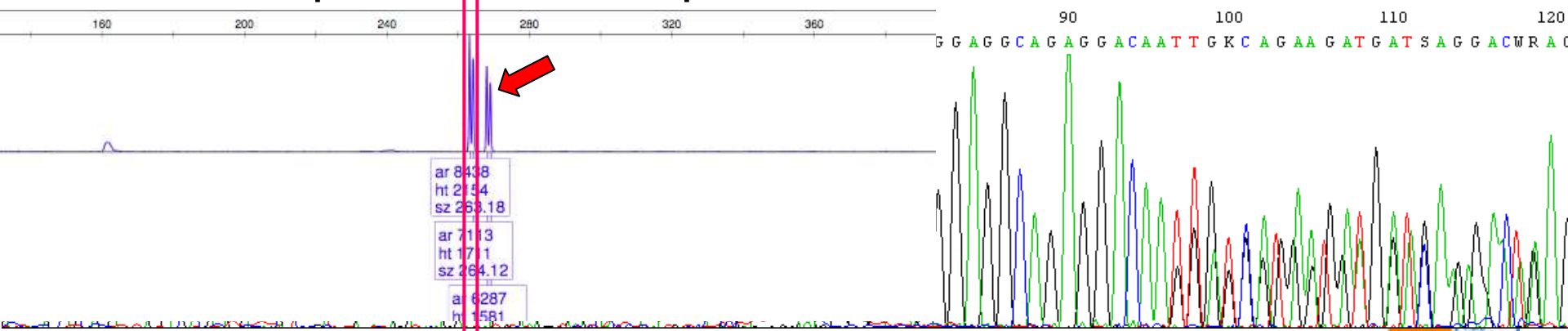




No mutada



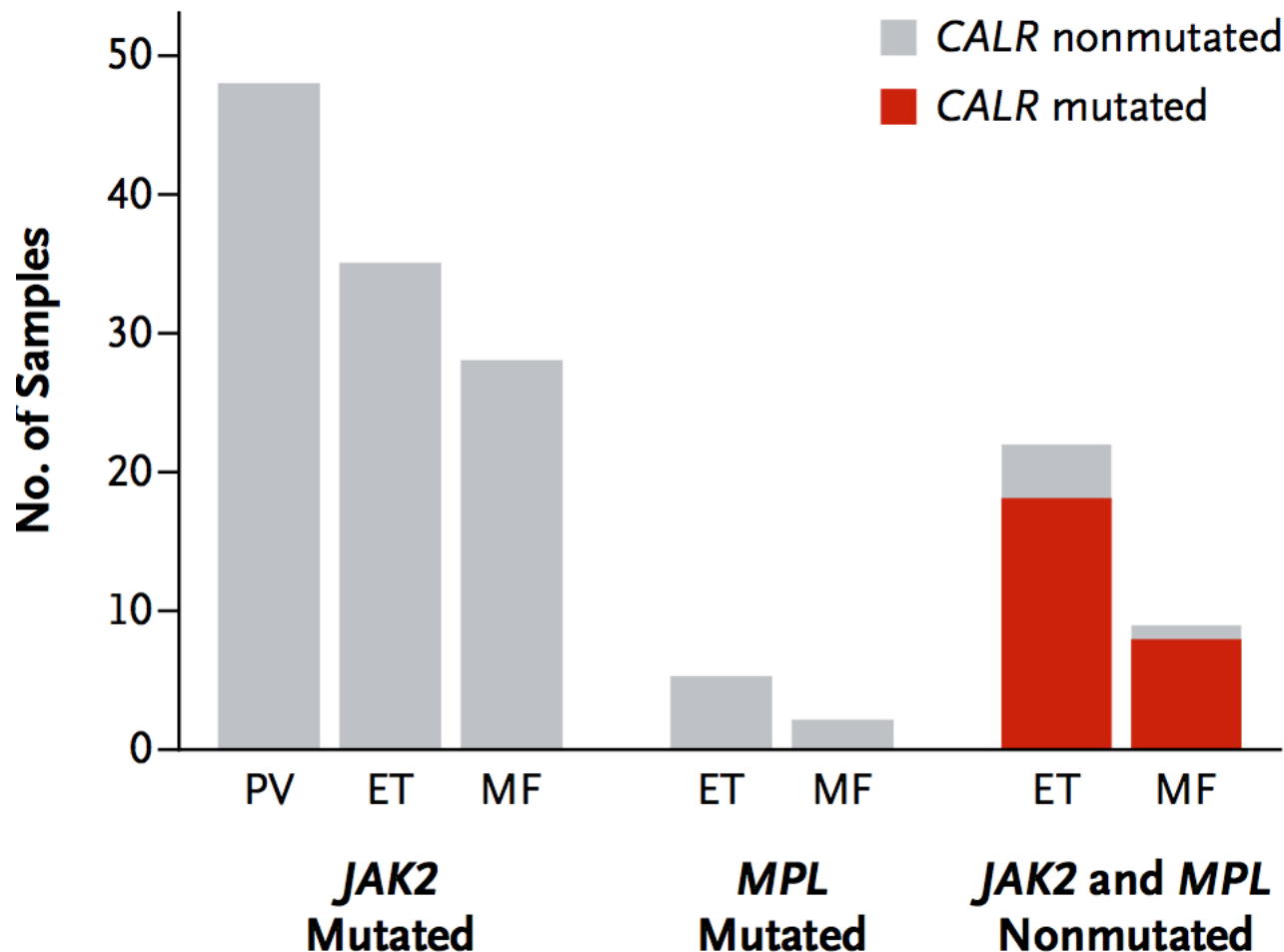
Delección 52 pb – mutación tipo 1



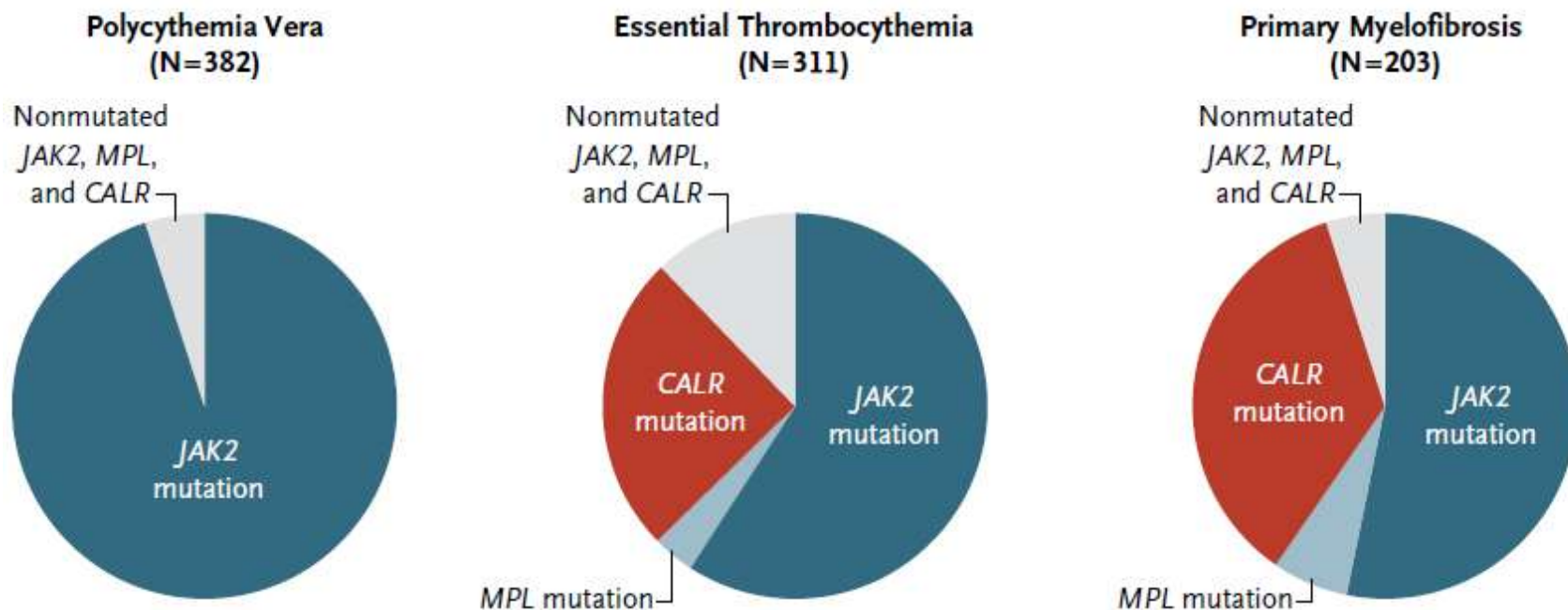
Inserción 5 pb – mutación tipo 2

Distribución de mutaciones en *CALR* en NM Ph negativas

***CALR* Mutations According to Diagnosis**



Distribución de mutaciones en *JAK2*, *MPL* y *CALR* en las NM Ph-negativas



Klampfl *et al*; NEJM 2013;369:2379

Pueden existir otras mutaciones que **coexisten** con *JAK2V617F*, *MPL* y *CALR*:
TET2, *ASXL1*, *DNMT3A*, *SF3B1*, *LNK*, *EZH2*, *CBL*, *IDH1/IDH2*

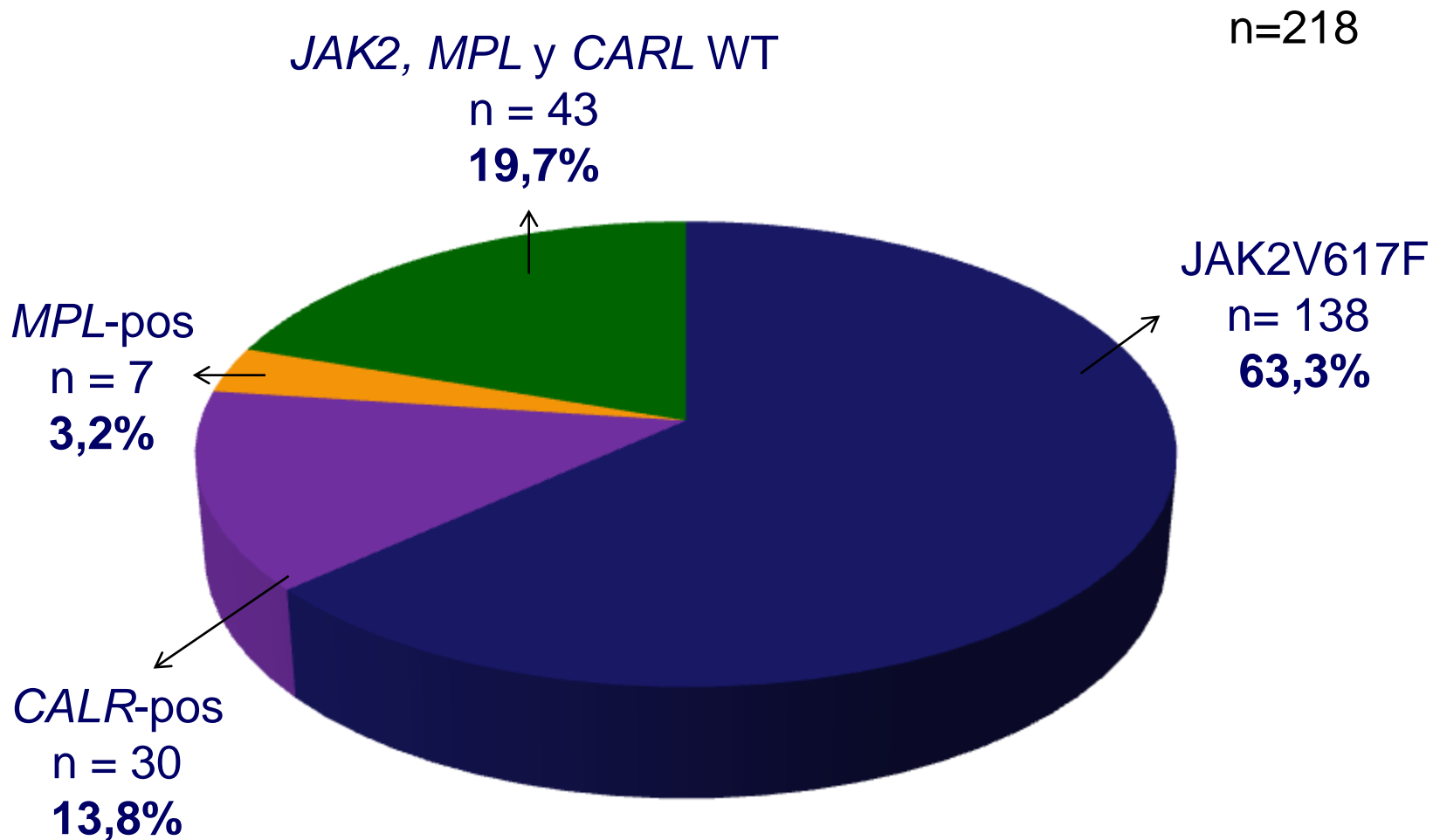
Mutaciones en *CALR* en pacientes con TE - Hospital del Mar

Cohorte 74 pacientes con TE , negativos para JAK2V617F y MPL

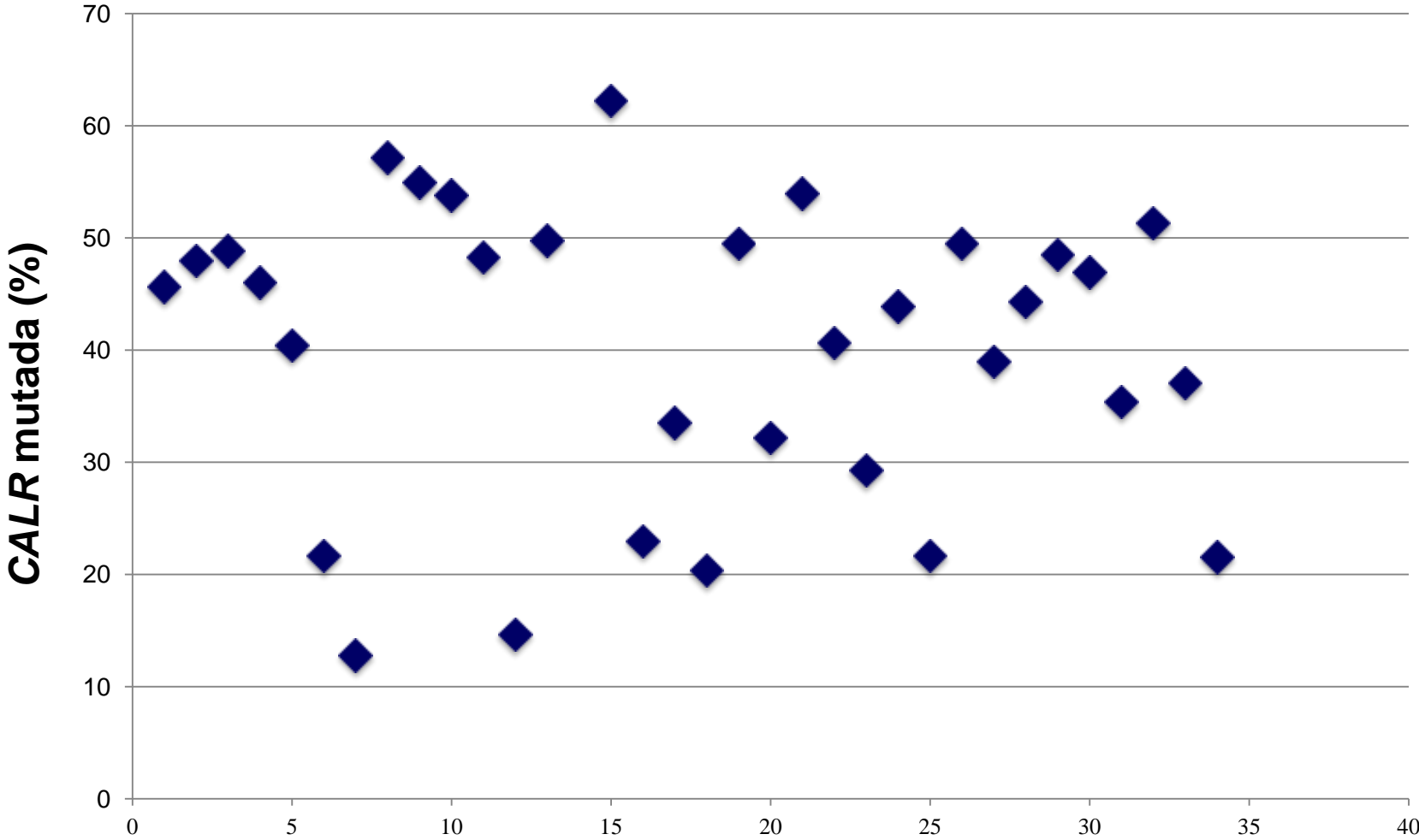
Mutación <i>CALR</i>	Nº pacientes
p.L367fs - (tipo 1; delección 52 bp)	14
p.K385fs - (tipo 2; inserción 5 bp)	12
p.E369fs (del 34)	1
p.E373fs (del 31)	1
p.K377fs (del 22)	1
p.K375fs (del 7 + ins 9)	1
p.K368fs (del 47 + ins T)	1
p.E383fs (del 6 + ins 5bp)	1
p.E370fs (del 1)	1
Total de pacientes con mutaciones en <i>CALR</i>	33

Marcadores moleculares en TE al diagnóstico

Experiencia del Hospital del Mar

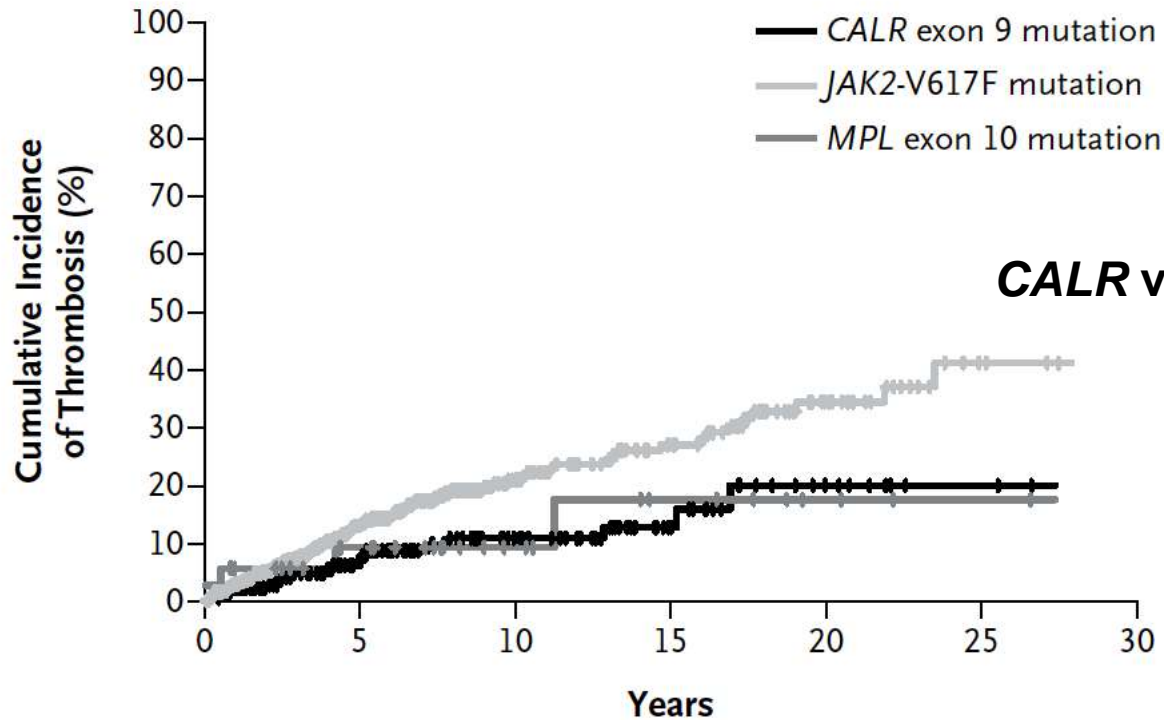


Mutaciones en *CALR* en pacientes con TE - Hospital del Mar



Las mutaciones en *CALR* se asocian a mejor pronóstico

C Thrombosis in Essential Thrombocythemia

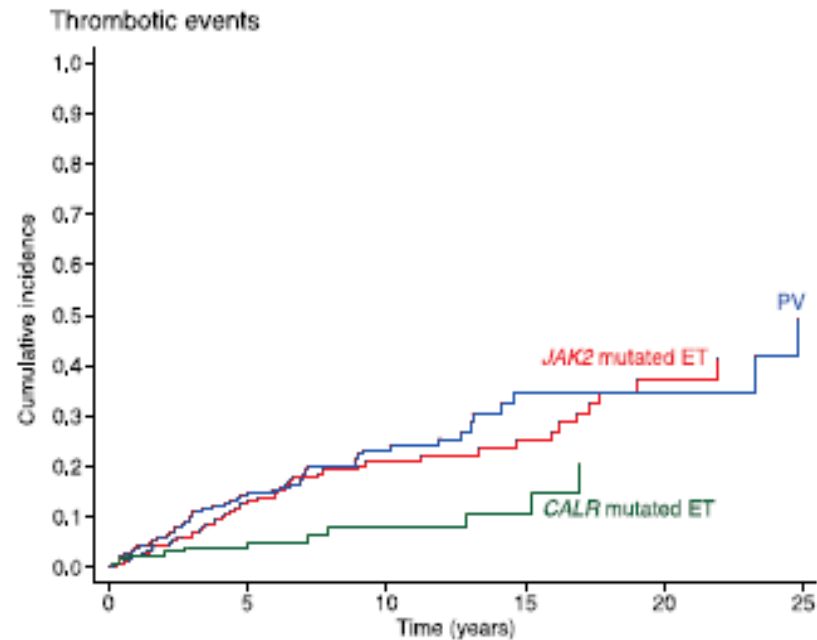
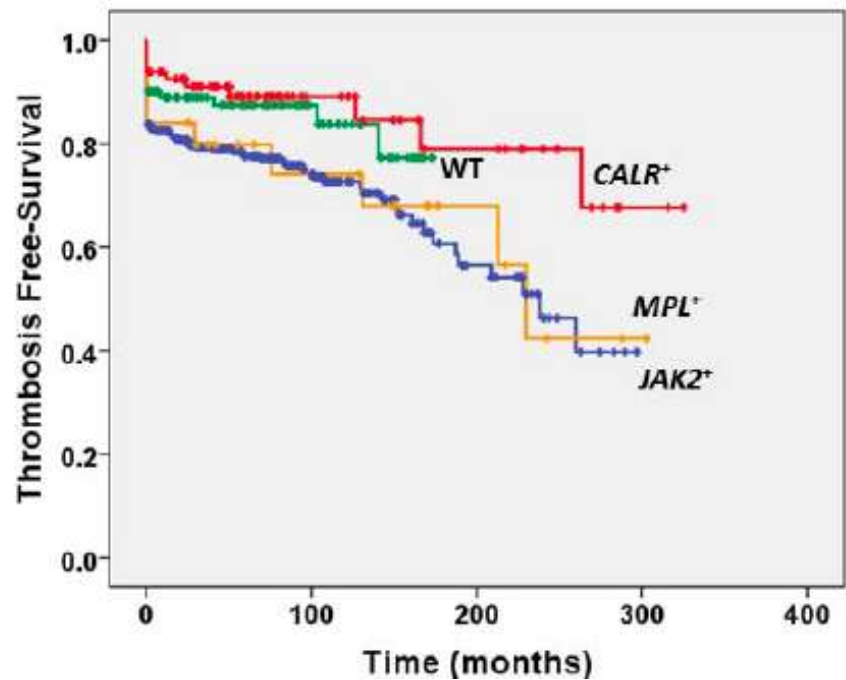


No. at Risk

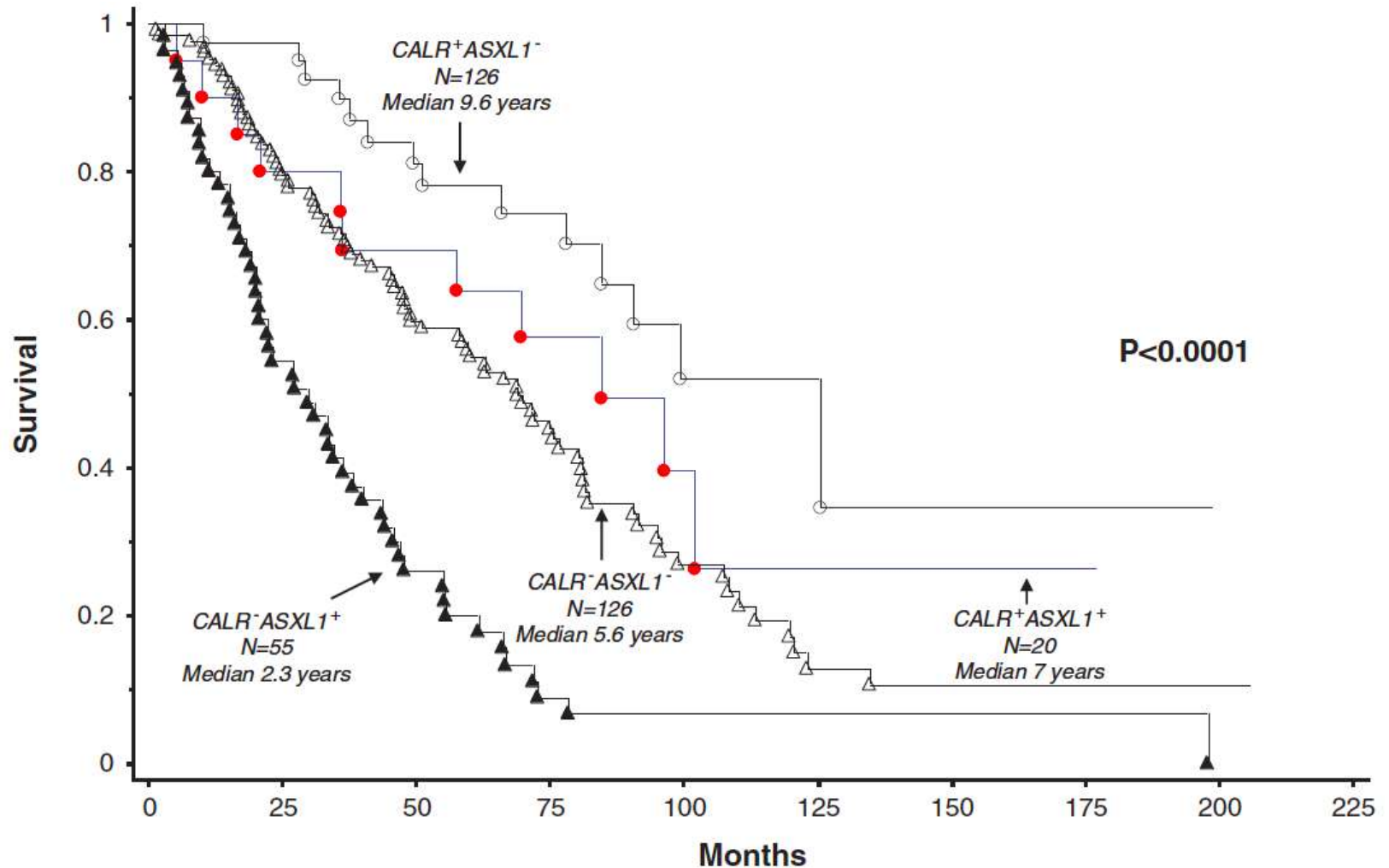
<i>CALR</i> mutation	186	115	63	27	9	3	0
<i>JAK2</i> mutation	575	267	116	62	25	5	0
<i>MPL</i> mutation	35	21	13	8	4	2	0

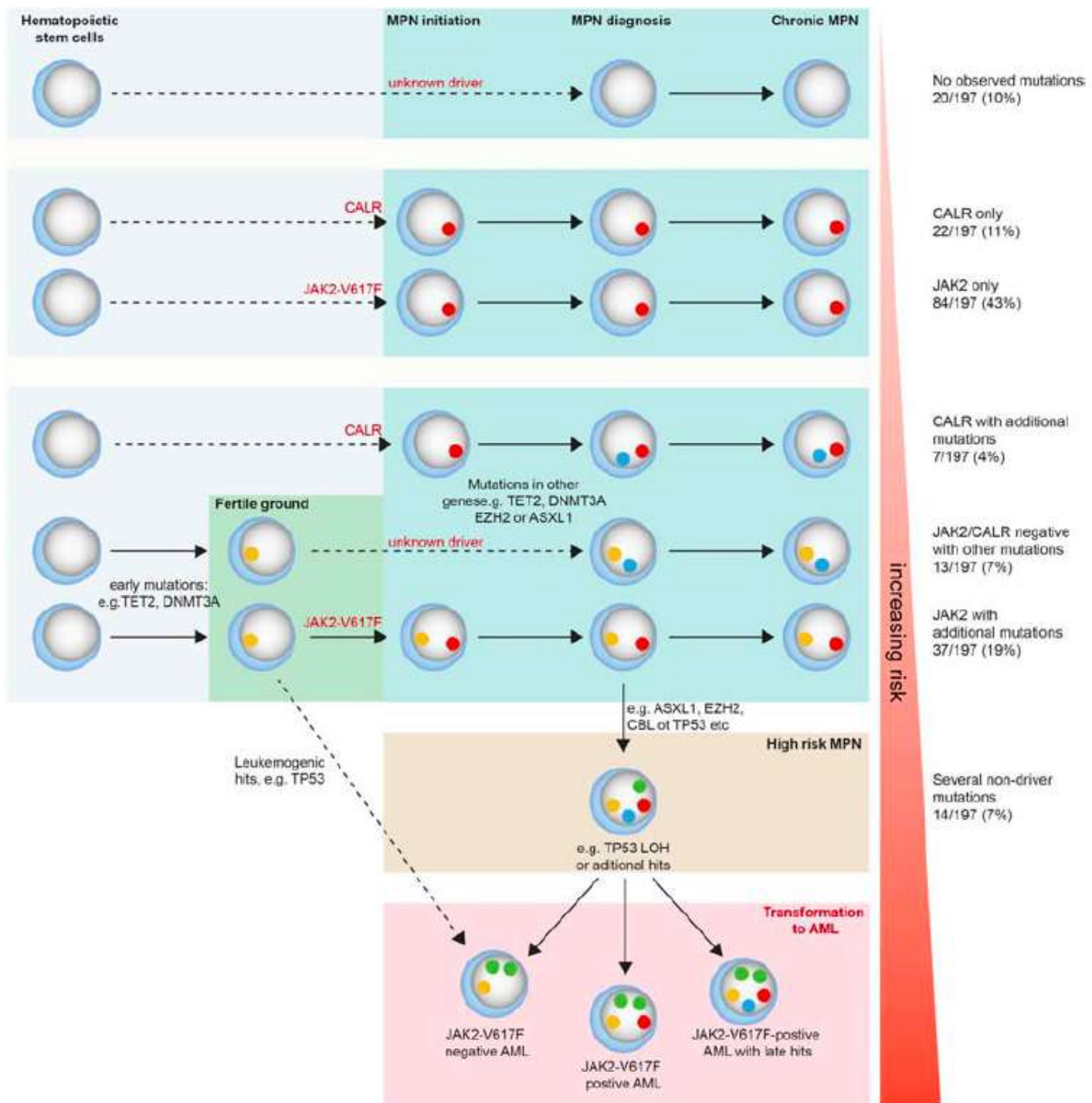
Las mutaciones en *CALR* se asocian a mejor pronóstico

A



Las mutaciones en *ASXL1* se asocian con mal pronóstico en MFP *CALR* negativa





Propuesta de revisión de criterios diagnósticos de las NMs

Table 4. 2014 proposed revision for World Health Organization (WHO) diagnostic criteria for *BCR-ABL1*-negative myeloproliferative neoplasms

<i>Polycythemia vera (PV)</i> ^a	<i>Essential thrombocythemia (ET)</i> ^b	<i>Primary myelofibrosis (PMF)</i> ^c
<i>Major criteria</i>		
1 Hemoglobin > 16.5 g/dl (men) > 16 g/dl (women) or hematocrit > 49% (men) > 48% (women)	Platelet count $\geq 450 \times 10^9/l$	Megakaryocyte proliferation and atypia ^d accompanied by either reticulin and/or collagen fibrosis or ^e
2 BM trilineage myeloproliferation with pleomorphic megakaryocytes	Megakaryocyte proliferation with large and mature morphology	Not meeting WHO criteria for CML, PV, ET, MDS or other myeloid neoplasm
3 Presence of <i>JAK2</i> mutation	Not meeting WHO criteria for CML, PV, PMF, MDS or other myeloid neoplasm	Presence of <i>JAK2</i> , <i>CALR</i> or <i>MPL</i> mutation
4	Presence of <i>JAK2</i> , <i>CALR</i> or <i>MPL</i> mutation	
<i>Minor criteria</i>		
1 Subnormal serum erythropoietin level	Presence of a clonal marker (e.g. abnormal karyotype) or absence of evidence for reactive thrombocytosis	Presence of a clonal marker (e.g. abnormal karyotype) or absence of evidence for reactive bone marrow fibrosis
2		Presence of anemia or palpable splenomegaly
3		Presence of leukoerythroblastosis ^f or increased lactate dehydrogenase ^f

Abbreviations: BM, bone marrow; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome. ^aPV diagnosis requires meeting either all three major criteria or the first two major criteria and one minor criterion. ^bET diagnosis requires meeting all four major criteria or first three major criteria and one minor criterion. ^cPMF diagnosis requires meeting all three major criteria or the first two major criteria and all three minor criteria. ^dSmall-to-large megakaryocytes with aberrant nuclear/cytoplasmic ratio and hyperchromatic and irregularly folded nuclei and dense clustering. ^eIn the absence of reticulin fibrosis, the megakaryocyte changes must be accompanied by increased marrow cellularity, granulocytic proliferation and often decreased erythropoiesis (that is, prefibrotic PMF). ^fDegree of abnormality can be borderline or marked and institutional reference range should be used for lactate dehydrogenase level.

¿Qué mutaciones génicas se deben determinar en los pacientes con NMs en el diagnóstico de rutina?

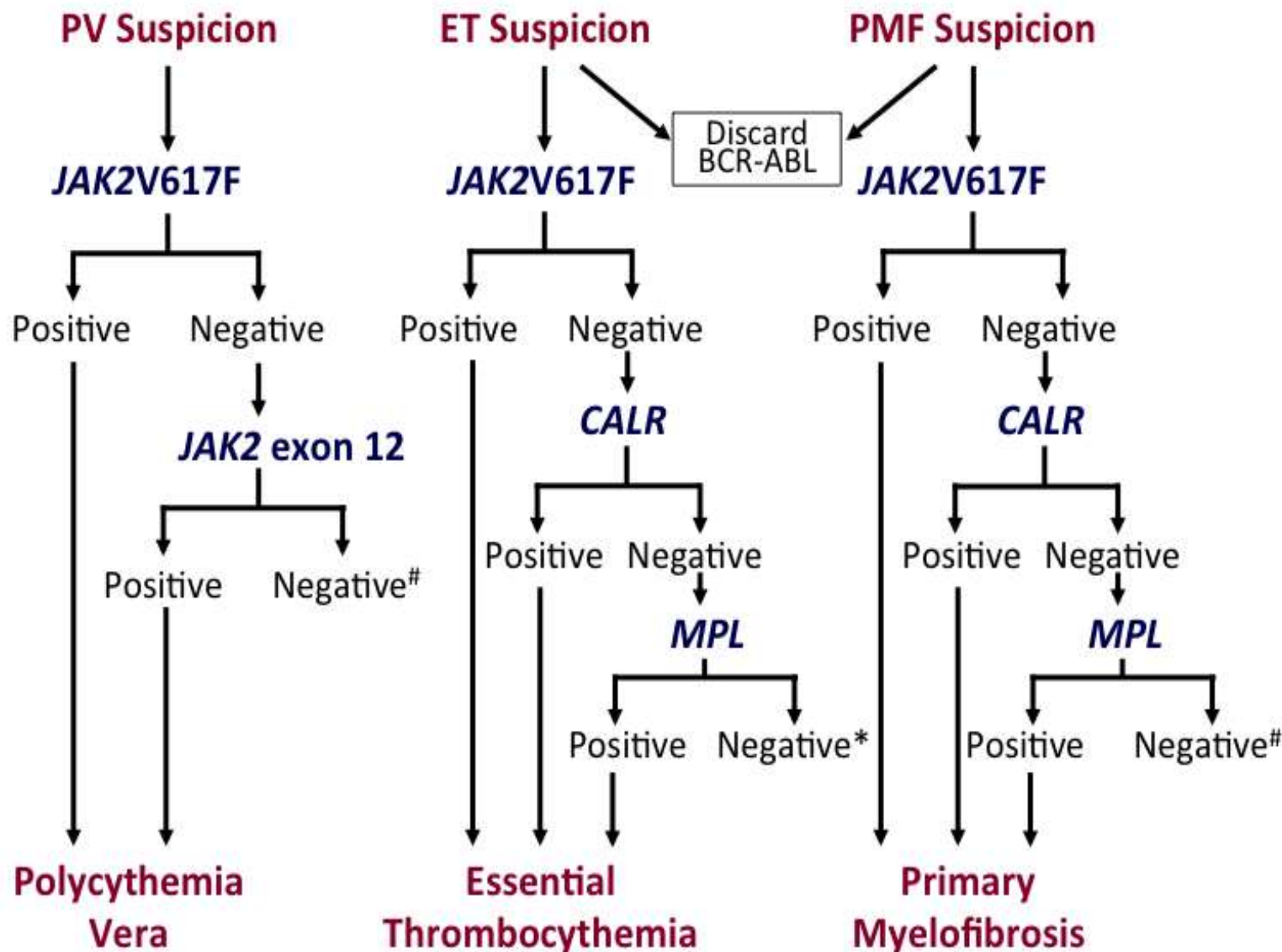
- **JAK2V617F**

- Todos los casos con sospecha de NM
- Es necesario un ensayo que detecte la mutación con una sensibilidad del 1-3%
- Un resultado positivo confirma el diagnóstico de NM, pero no informa del subtipo
- Un resultado negativo **no excluye** el diagnóstico de una NM

¿Qué mutaciones génicas se deben determinar en los pacientes con NMs en la rutina diagnóstica?

- **JAK2 - mutaciones exón 12**
 - Sólo en los casos *JAK2V617F* negativos con evidencia clínica de PV
- **CALR mutaciones exón 9**
 - Sospecha de MFP o TE en pacientes negativos para *JAK2V617F*
- **MPL mutaciones exón 10**
 - Sospecha de MFP o TE en pacientes negativos para *JAK2V617F* y *CALR*
- **TET2, ASXL1, EZH2, SRSF2, SF3B1, etc**
 - Su papel diagnóstico/pronóstico está por determinar

¿Qué mutaciones génicas se deben determinar en los pacientes con NMs en la rutina diagnóstica?





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