SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA
Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori
Istituto di Ricovero e Cura a Carattere Scientifico

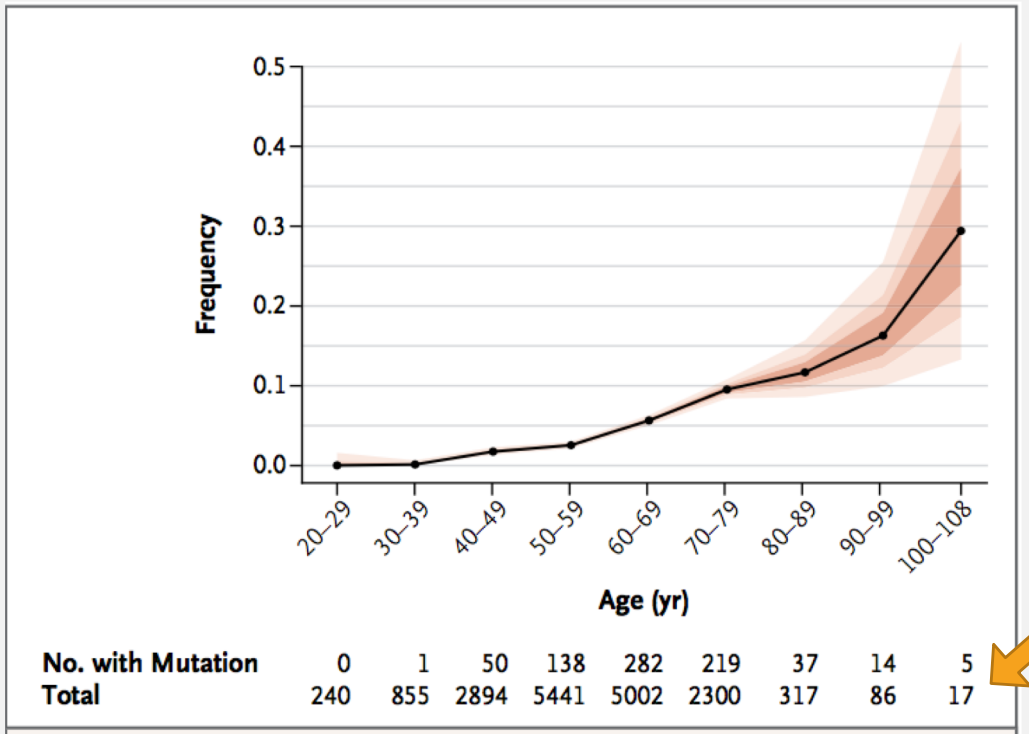
ISTITUTO SCIENTIFICO ROMAGNOLO PER LO STUDIO E LA CURA DEI TUMORI

PUBLIC
PRIVATE
PARTNERSHIP

Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori
IRST-IRCCS

Prof. Giovanni Martinelli

Mutational Frequency and Age



No. with Mutation	0	1	50	138	282	219	37	14	5
Total	240	855	2894	5441	5002	2300	317	86	17

Figure 1. Prevalence of Somatic Mutations, According to Age.

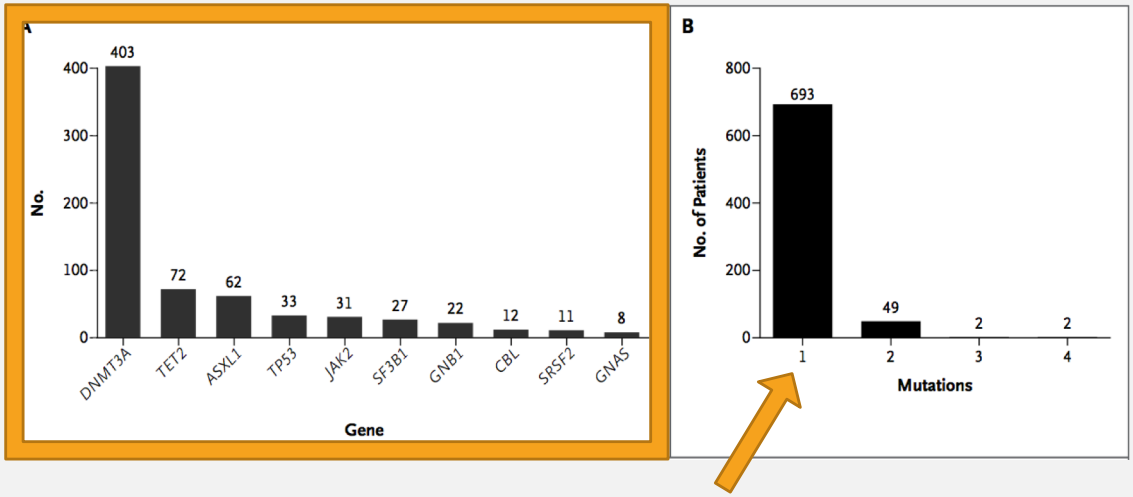
Colored bands, in increasingly lighter shades, represent the 50th, 75th, and 95th percentiles.

17 persons aged between 100-108 **5 with a mutation**

1. *cardiovascular risk*

Somatic Mutations

- Most frequently mutated genes:
  - **DNMT3A**
  - **TET2**
  - **ASXL1**
  - TP53
  - JAK2
  - SF3B1

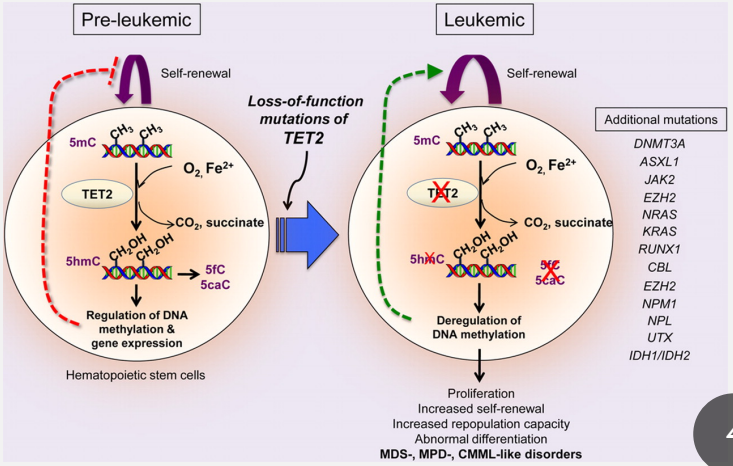


- The majority of pts (693 of 746) have **only 1 mutation**
  - consistent with the hypothesis that these persons had clones that harbor only initiating lesions

1. *cardiovascular risk*

TET2

- **TET2**
  - the first gene reported to exhibit somatic mutations in blood cells in individual with clonal hematopoiesis and w/o hematological malignancies
  - is frequently mutated in myeloid malignancies (MDS, AML, CMML)
- **Tet2**: epigenetic regulatory enzyme
  - Oxidation of 5-methylcytosine (5mC) in DNA in 5-hydroxymethylcytosine (5hmC)
  - Non catalytic f(x)s
- Role in the self renewal of the hematopoietic stem cell
- Role in CVD unexplored



TET2: epigenetic safeguard for HSC, Myunggon Ko, Blood 2011

1. *cardiovascular risk*

To sum up

- **Tet2 deficiency** induce clonal hematopoiesis in mice
- That is associated with *increased atherosclerotic plaque size*
- Tet2 deficient macrophages produce more **IL-1β** due to **NLRP3** inflammasome pathway
- NLRP3 inflammasome inhibitor reduce the size of the plaque (new therapeutic target?)

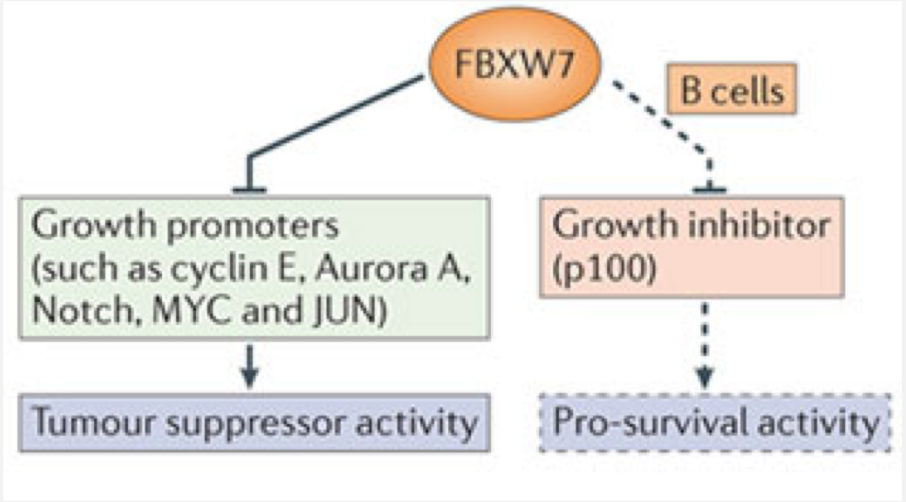
FBXW7

- CAVE: Mutation seen also by Jaiswal et al in Age-related Clonal Hematopoiesis Associated with Adverse Outcomes [NEJM 2014] from supplementary

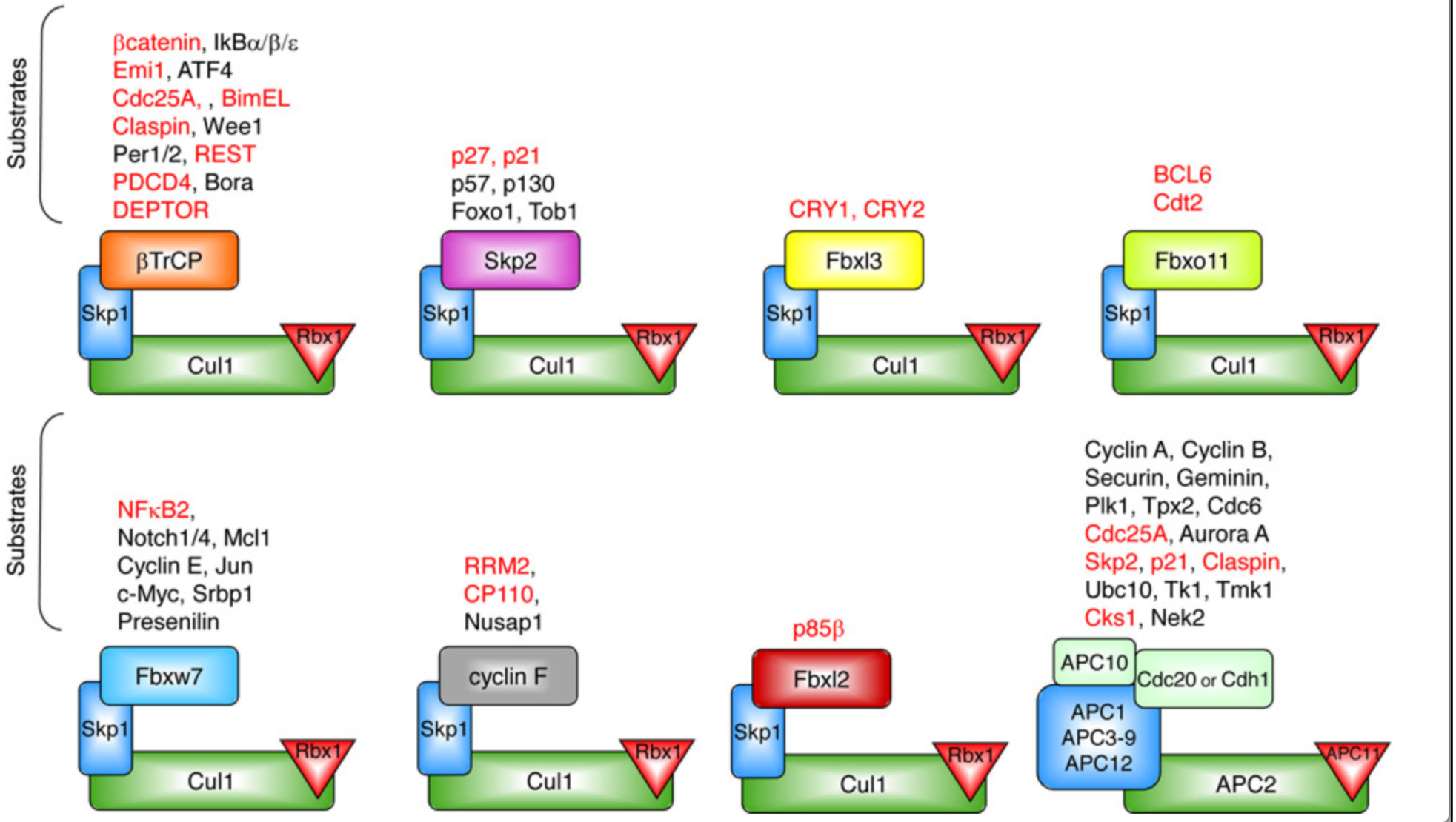
FBXO11	Frameshift/nonsense/splice-site	NM_001190274	0
FBXW7	Frameshift/nonsense/splice-site, E74A, D101V, F280L, R465H, R505C, G597E, R1165Q	NM_033632	1
FLT3	V579A, V592A, V592I, F594L, M737I, FY590-591GD	NM_004119	1
FOXP1	Frameshift/nonsense/splice-site	NM_032682	1
FYN	L174R, R176C, Y531H	NM_002037	0
GATA1	Frameshift/nonsense/splice-site	NM_002049	0
GATA2	Frameshift/nonsense/splice-site, R293Q, N317H, A318T, A318V, A318G, G320D, L321P, L321F, L321V, Q328P, R330Q, R361L, L359V, A372T, R384G, R384K	NM_001145661	0
GATA3	Frameshift/nonsense/splice-site ZNF domain, R276W, R276Q, N286T, L348V,	NM_001002295	0
GNA13	I34T, G57S, S62F, M68K, Q134R, Y145F, L152F, E167D, Q169H, R264H, E273K, V322G, V362G, L371F	NM_006572	0
GNAS	R201(844)S, R201(844)C, R201(844)H, R201(844)L, Q227(870)K, Q227(870)R, Q227(870)L, Q227(870)H, R374(1017)C	NM_016592	8
GNB1	K57N, K57M, K57E, K57T, I80T, I80N	NM_002074	22
HIST1H1B	S89N, S89R, G101D, G73A, K84N, A123D	NM_005322	0
HIST1H1C	P118S, P129A, K156R, K187R, K/G81/83N/A	NM_005319	1

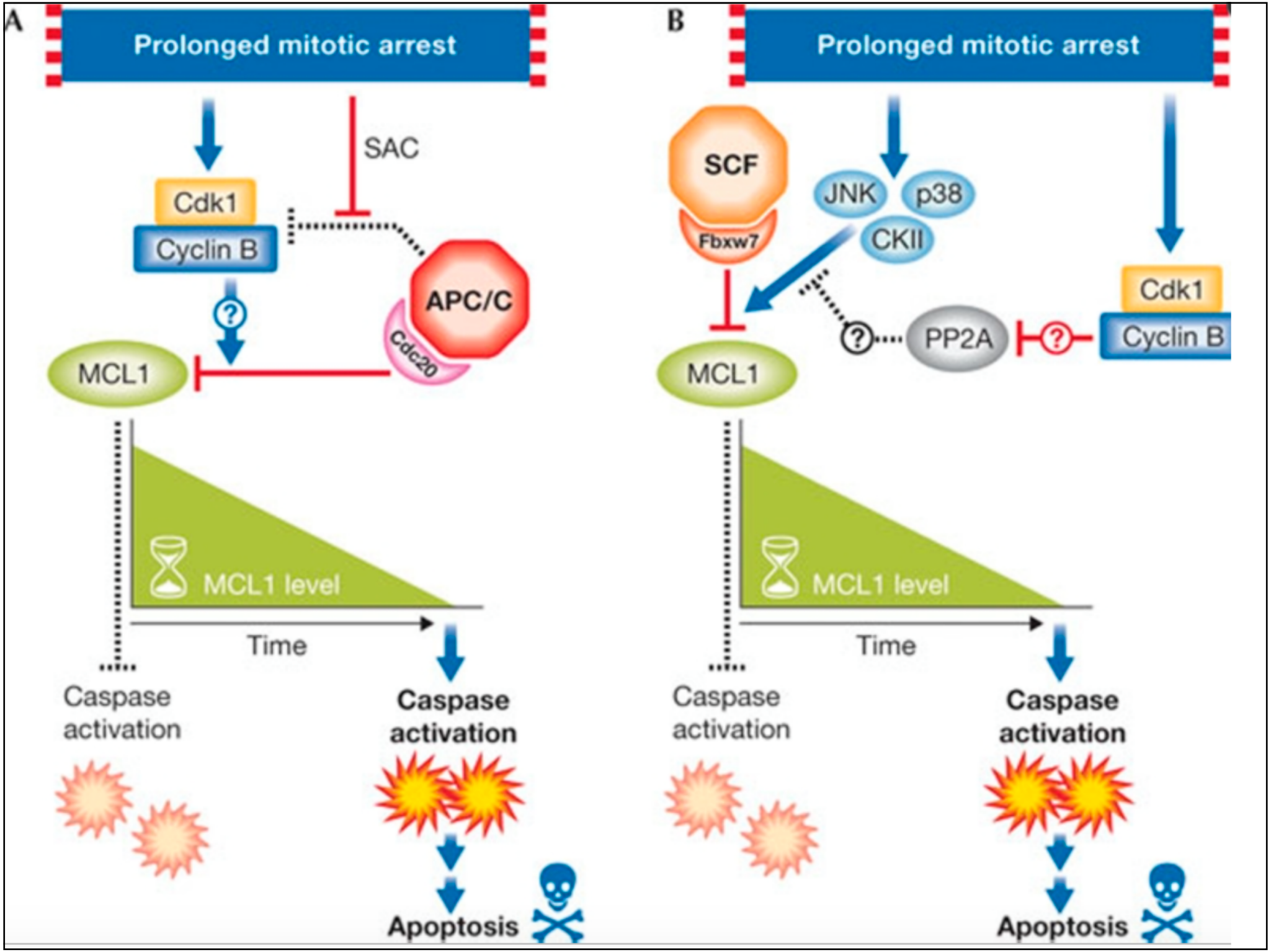
FBXW7

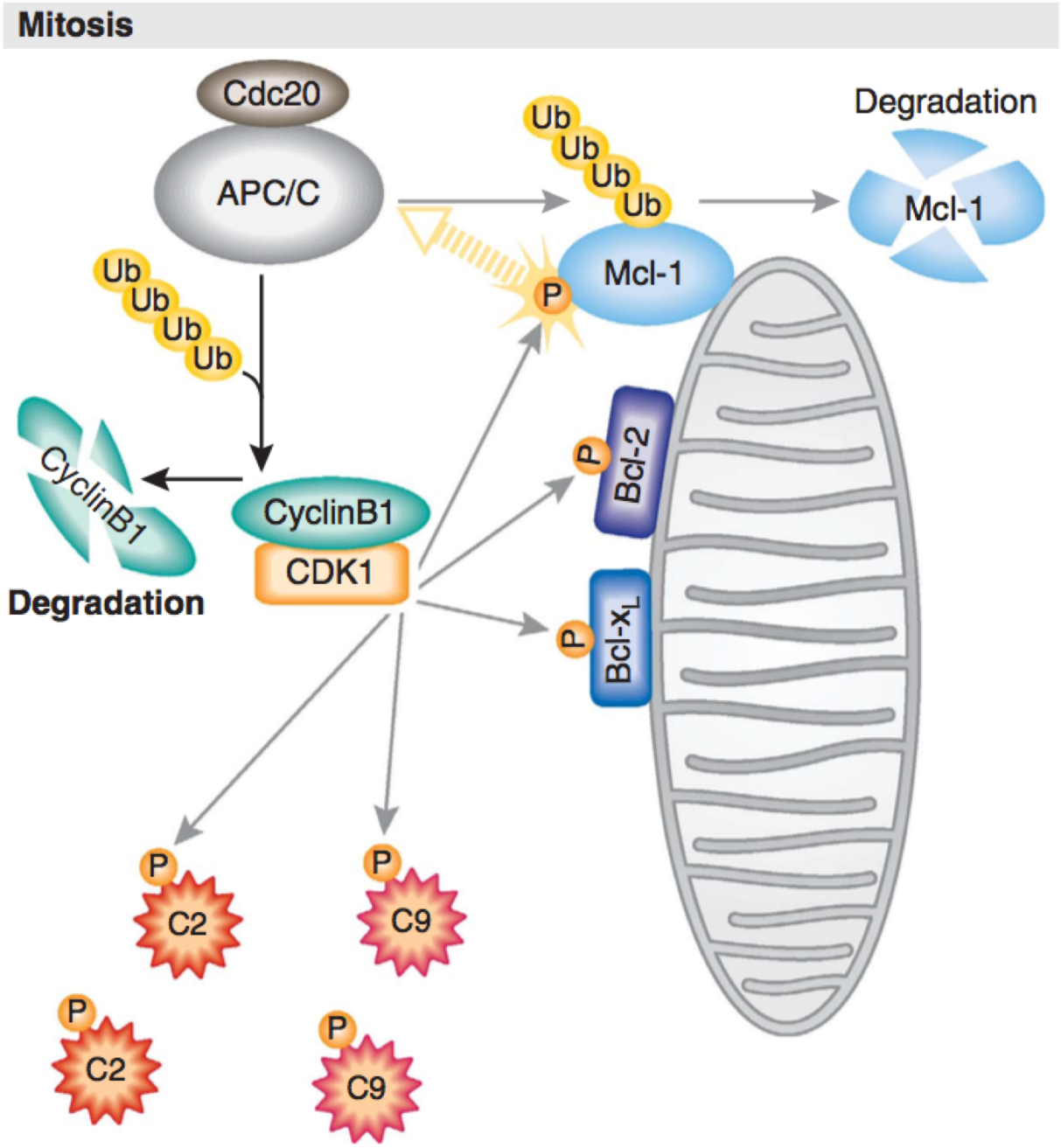
- Ubiquitin ligase of F-box protein family
- In mouse models, FBXW7 functions as a **tumour suppressor** by ubiquitylating growth-promoting substrates, but this role is cell type specific.
- In B cell lineages, FBXW7 actually has a **pro-survival role** by mediating the degradation of p100, an inhibitor of nuclear factor-κB (NF-κB) signalling



Mechanisms and function of substrate recruitment by F-box proteins,
Jeffrey R. Skaar, *Nature Reviews Molecular Cell Biology 2013*


Substrates
βcatenin, IkBα/β/ε
Emi1, ATF4
Cdc25A, , BimEL
Claspin, Wee1
Per1/2, REST
PDCD4, Bora
DEPTOR
βTrCP
Skp1
Cul1
Rbx1
p27, p21
p57, p130
Foxo1, Tob1
Skp2
Skp1
Cul1
Rbx1
CRY1, CRY2
Fbxl3
Skp1
Cul1
Rbx1
BCL6
Cdt2
Fbxo11
Skp1
Cul1
Rbx1
Substrates
NFκB2,
Notch1/4, Mcl1
Cyclin E, Jun
c-Myc, Srbp1
Presenilin
Fbxw7
Skp1
Cul1
Rbx1
RRM2,
CP110,
Nusap1
cyclin F
Skp1
Cul1
Rbx1
p85β
Fbxl2
Skp1
Cul1
Rbx1
Cyclin A, Cyclin B,
Securin, Geminin,
Plk1, Tpx2, Cdc6
Cdc25A, Aurora A
Skp2, p21, Claspin,
Ubc10, Tk1, Tmk1
Cks1, Nek2
APC10
Cdc20 or Cdh1
APC1
APC3-9
APC12
APC2
APC11


A
Prolonged mitotic arrest
SAC
Cdk1
Cyclin B
APC/C
Cdc20
MCL1
MCL1 level
Time
Caspase activation
Caspase activation
Apoptosis
B
Prolonged mitotic arrest
SCF
Fbxw7
JNK
p38
CKII
Cdk1
Cyclin B
PP2A
MCL1
MCL1 level
Time
Caspase activation
Caspase activation
Apoptosis


Mitosis
Cdc20
APC/C
Ub
Ub
Ub
Ub
Degradation
Mcl-1
P
Mcl-1
Ub
Ub
Ub
Ub
CyclinB1
Degradation
CyclinB1
CDK1
P
Bcl-2
P
Bcl-xL
P
C2
P
C9
P
C2
P
C9

3. Clonal hematopoiesis: **MDS and anemia in the elderly**

New challenges in evaluating anemia in older persons in the era of molecular testing [D Steensma, ASH 2016]

Hematology Am Soc Hematol Educ Program. 2016 Dec 2;2016(1):67-73.

New challenges in evaluating anemia in older persons in the era of molecular testing.

Steensma DP[1].

Author information

Abstract

Anemia is common in older persons, and often remains unexplained despite a thorough clinical history, physical examination, and focused laboratory testing, including marrow aspiration, biopsy, and karyotyping. The advent of molecular genetic testing panels in hematology clinical practice has complicated the evaluation of older patients with unexplained anemia. While the presence of a somatic mutation provides evidence of clonal hematopoiesis and may support a diagnosis of a hematologic neoplasm such as one of the myelodysplastic syndromes (MDS), with rare exceptions, individual mutations are not strongly associated with one specific diagnosis, nor are they by themselves diagnostic of neoplasia. A clonal mutation in a patient with cytopenias and a nondiagnostic bone marrow may indicate a syndrome with a similar natural history to MDS, but at present there are no clear criteria to distinguish cytopenias coincidentally seen in association with an unrelated clonal mutation from cytopenias that are directly caused by that mutation. Ongoing and planned analyses will help define when mutation patterns alone can identify a disorder equivalent to a morphologically defined myeloid neoplasm such as MDS, further clarifying the etiology and natural history of unexplained anemia in the elderly.

PMID: 27913464 DOI: 10.1182/asheducation-2016.1.67

[PubMed - in process]

- Presence of somatic mutation provides evidence of clonal hematopoiesis and may support diagnosis of MDS (also when there is a non-diagnostic bone marrow nor classical cytogenetic alterations)

Anemia in older persons

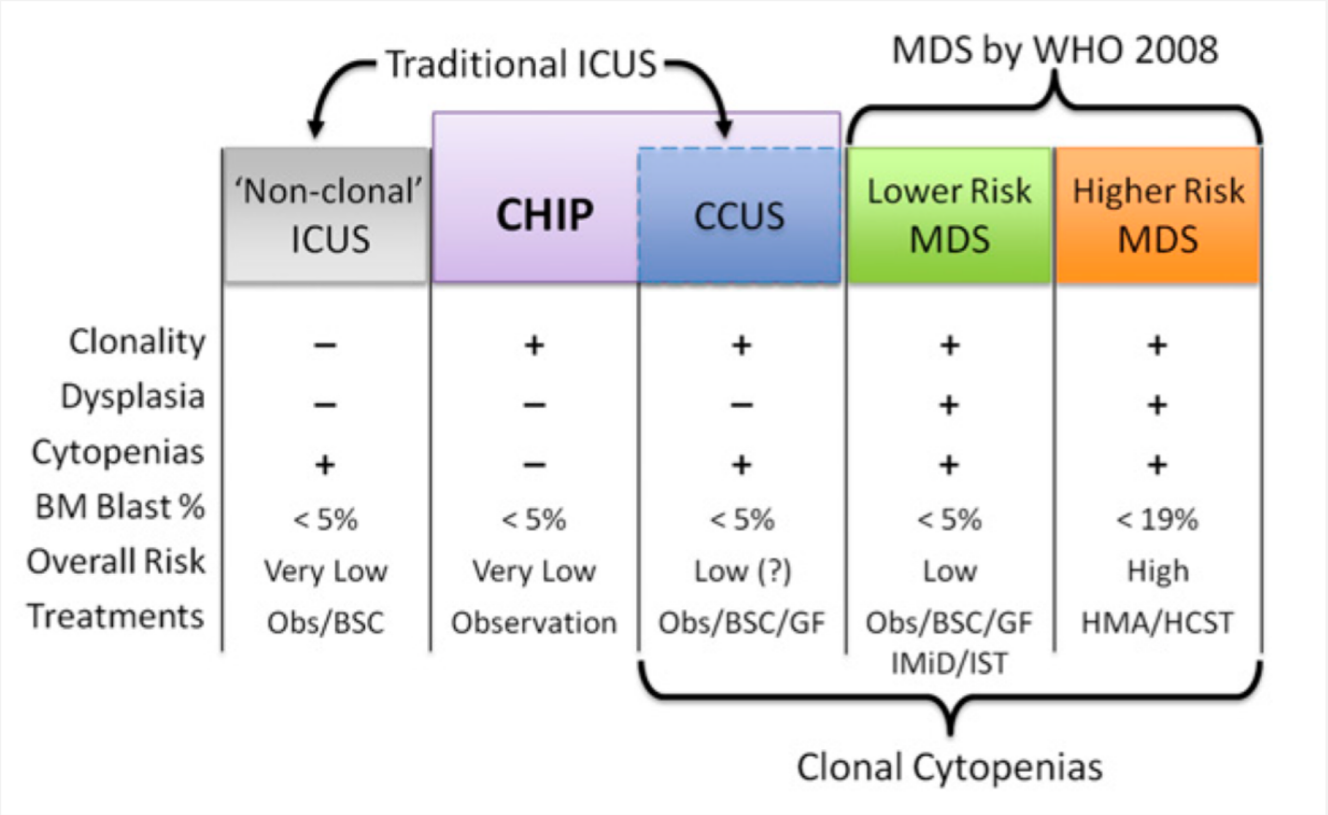
- NHANES III (1988 –1994): 11% of men and 10.2 % of women older than 65 years old were anemic
- Leading causes:
  - Nutritional deficiency (Iron, B12, folate)
  - Inflammation (driven by hepcidin)
  - Erythropoietin (EPO) deficiency caused by chronic kidney disease (when EGFR falls below 40 mL/min)
- Anemia itself is a risk factor associated with many complications including geriatric health problems such as
  - frailty,
  - cognitive dysfunction
  - falls

Unexplained Anemia

- Unexplained anemia mechanisms proposed:
  - low testosterone
  - Progressive stem cells dysfunction
  - Acquired resistance to EPO

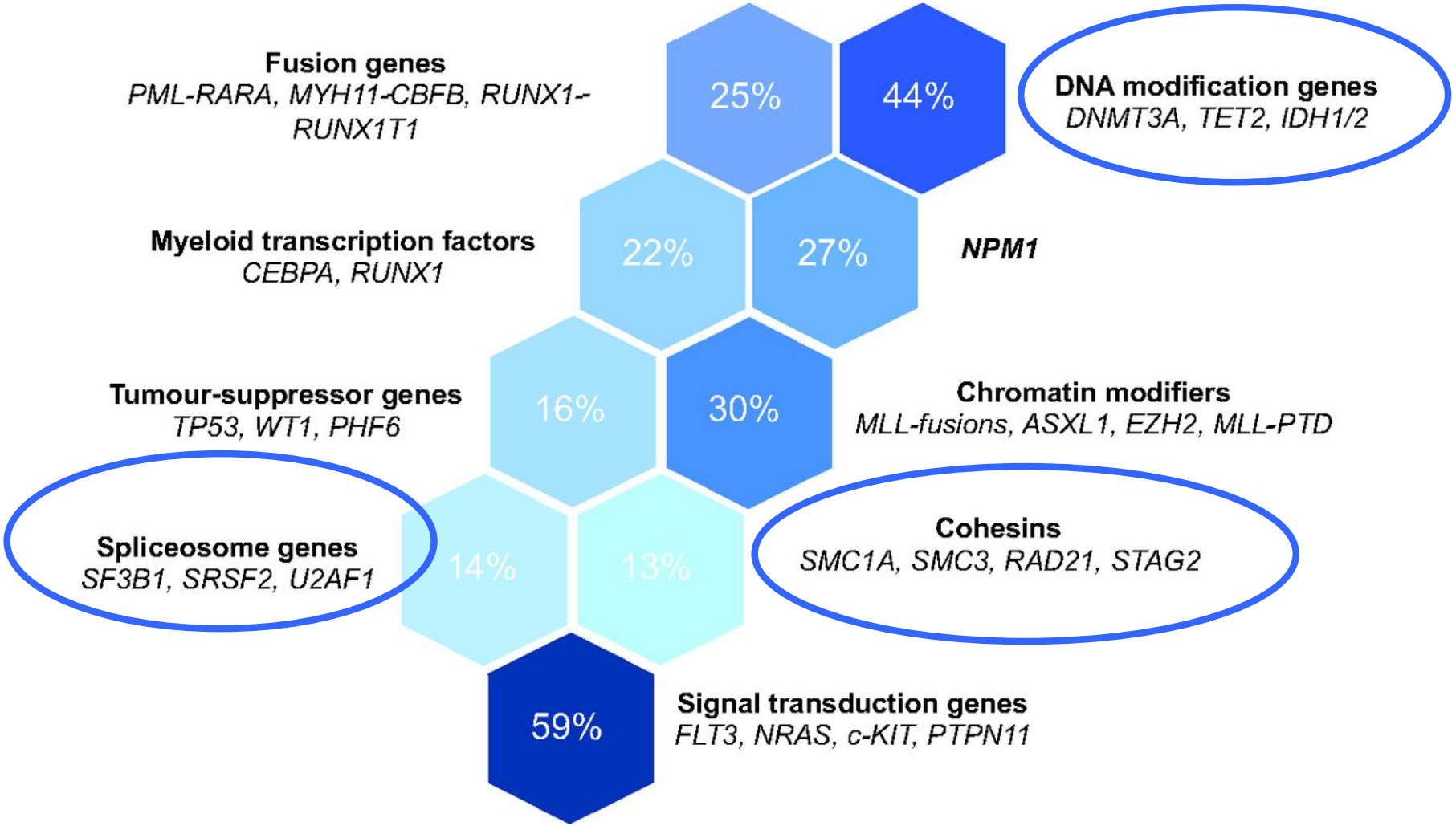
- NEW ENTITIES:
  - ICUS: idiopathic cytopenia of undetermined significance
  - CCUS: clonal cytopenia of undetermined significance
  - CHIP: clonal hematopoiesis of indeterminate potential

MDS and CHIP: what's the difference



3. *MDS and anemia in the elderly*

Recurrent mutation groups in secondary (MDS) and de novo AML: distinct functional groups or pathways



Carolyn S. Grove, and George S. Vassiliou Dis. Model. Mech. 2014;7:941-951

Actionable molecular targets in MDS …

ABL1		***HRAS***	***MYD88***	*SF3B1*
ASXL1	*CSF3R*	***IDH1***	***NOTCH1***	*SMC1A*
ATRX	*CUX1*	***IDH2***	*NPM1*	*SMC3*
BCOR	***DNMT3A***	***IKZF1***	***NRAS***	*SRFS2*
BCORL1	*ETV6*	***JAK2***	***PDGFRA***	*STAG2*
BRAF		*JAK3*	*PHF6*	*TET2*
CALR		*KDM6A*	*PTEN*	***TP53***
CBL	***FLT3***	***KIT***	*PTPN11*	*U2AF1*
CBLB	*GATA1*	***KRAS***	*RAD21*	*WT1*
CBLC	*GATA2*	*MLL*	*RUNX1*	*ZRSR2*
CDKN2A	*GNAS*	*MPL*	*SETBP1*	

AG120

AG221

Azacitidine
Decitabine

Idasanutlin
Decitabine

Healthcare Alliance for Resourceful Medicines Offensive against Neoplasms in HematologY

Action Acronym: **HARMONY**

HARMONY

The HARMONY consortium

HARMONY is a European Network of Excellence that captures, integrates, analyzes and harmonizes big data from high-quality multidisciplinary sources with the purpose of unlocking valuable knowledge on various hematologic malignancies (HMs).

Myeloid pannel and MRD assessment

Ngs Lab Service at IRCCS

ISTITUTO SCIENTIFICO ROMAGNOLO PER LO STUDIO E LA CURA DEI TUMORI

Dipartimento di Oncologia ed Ematologia Clinica e Sperimentale
SS Diagnostica Molecolare Avanzata e Predittiva

IRST - Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) S.r.l. IRCCS
Via Piero Maroncelli, 40 - 47014 Meldola (FC), Italy

SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA
Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori
Istituto di Ricovero e Cura a Carattere Scientifico

ISTITUTO SCIENTIFICO ROMAGNOLO PER LO STUDIO E LA CURA DEI TUMORI

Tipo di cancro del campione: Cancro ovarico

Indice

Aspetti principali del report

1 Varianti driver
1 Terapie disponibili
10 Studi clinici

Riepilogo delle varianti

Indicata Controindicata

Alterazione genomica	Terapie disponibili (In questo tipo di cancro)	Terapie disponibili (In un altro tipo di cancro)	Studi clinici
BRCA2 mutation	olaparib[1]	Nessuna	10

Fonti incluse nelle terapie disponibili: EMA[1], ESMO

Riepilogo delle varianti

Indicata ■ Controindicata

Alterazione genomica	Terapie disponibili (In questo tipo di cancro)	Terapie disponibili (In un altro tipo di cancro)	Studi clinici
BRCA2 mutation	olaparib[1]	Nessuna	10

Fonti incluse nelle terapie disponibili: EMA[1], ESMO

Riepilogo della terapia pertinente

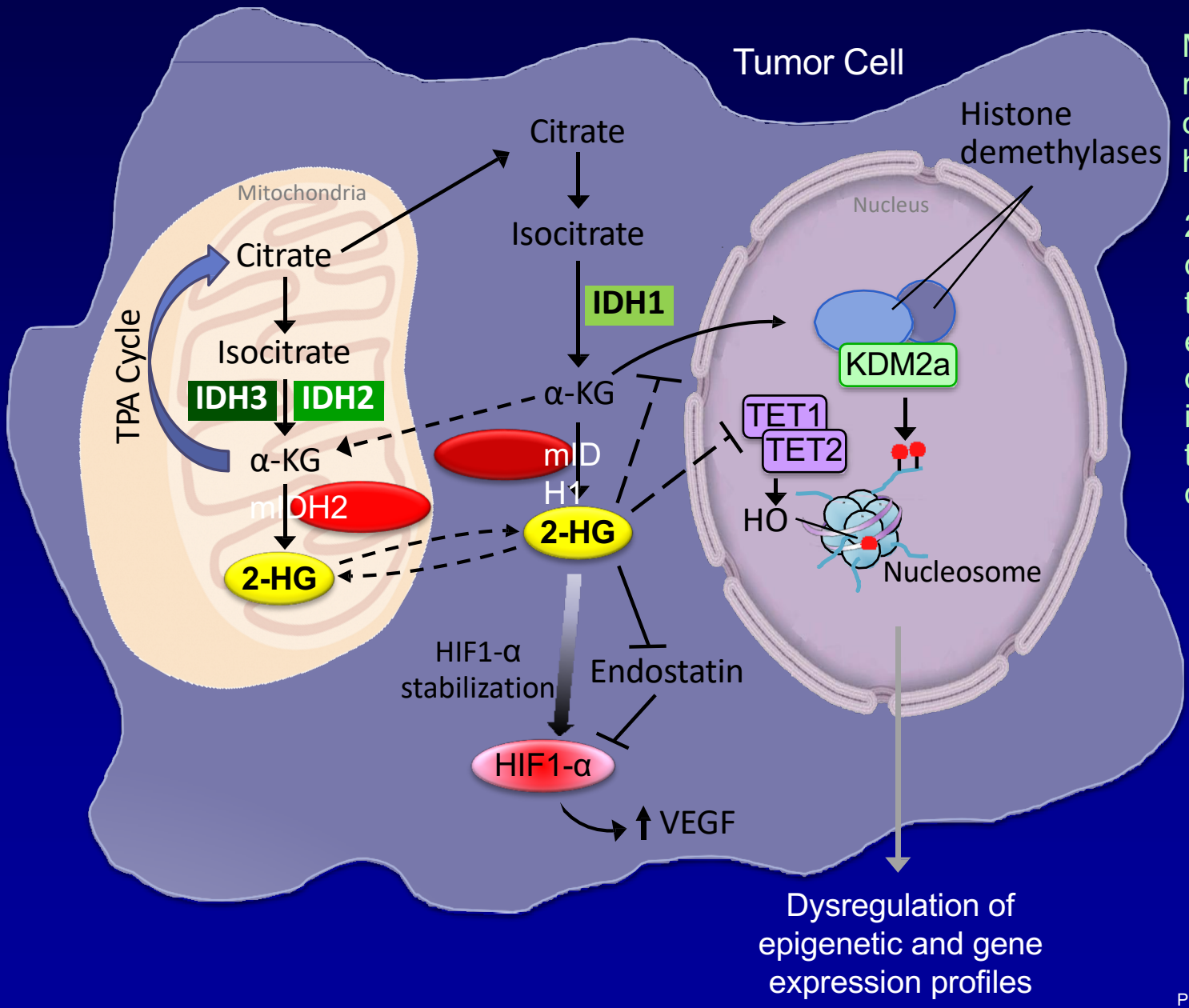
● In questo tipo di cancro ○ In un altro tipo di cancro ◑ In questo tipo di cancro e in altri tipi di cancro ⊘ Controindicato ⚠ Sia per l'uso che controindicato ✖ Nessuna evidenza

BRCA2 mutation

Terapia pertinente	EMA	ESMO	Studi clinici*
olaparib	●	●	● (IV)
niraparib	✖	✖	● (III)
rucaparib	✖	✖	● (III)
cediranib + olaparib, olaparib	✖	✖	● (II)
olaparib + chemotherapy	✖	✖	● (I/II)
VX-970, VX-970 + chemotherapy	✖	✖	● (I/II)
atezolizumab + rucaparib	✖	✖	● (I)
BAY-1895344	✖	✖	● (I)

* Viene mostrata la fase più avanzata (IV, III, II/III, II, I/II, I) e possono essere disponibili più studi clinici.

IDH mutations in MDS and Leukemia and Cancer



Mutant IDH1 and IDH2 results in an increase of the oncometabolite, 2-hydroxyglutamate (2-HG)

2-HG induces a block of cell differentiation by inhibiting the chromatin-modifying enzymes, DNA and histone demethylases, which results in hypermethylated DNA, thereby blocking cell differentiation

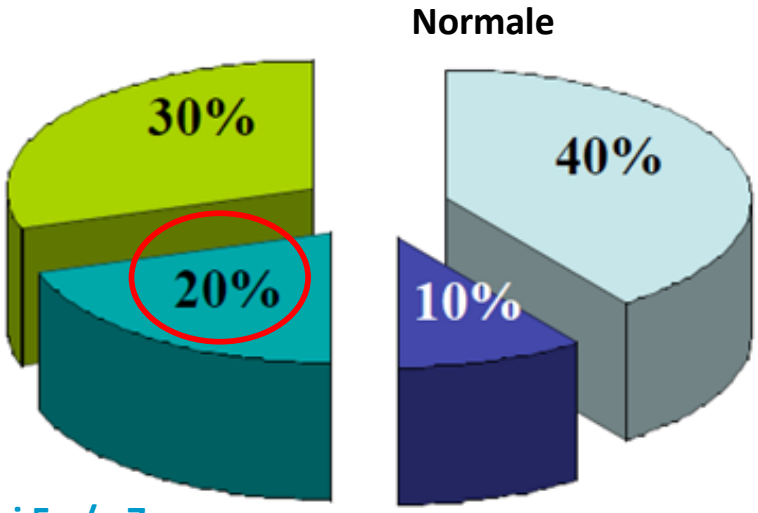
TET2 is an α-KG-dependent dioxygenase that is inhibited by 2-HG
TET2 is thought to be involved in both passive and active DNA demethylation

Prensner & Chinnaiyan. *Nature Med* 2011;17:291-3
Schoofs et al. *Leukemia* 2014;28:1-14

Alterazioni citogenetiche e SMD

Altre sbilanciate
-17/17p-
der/del(11q)
del(12p)
del(13q)/ -13
iso17q
del(9q)
Trisomia 8
Del20q
Monosomia Y
Trisomia 11
Trisomia 21

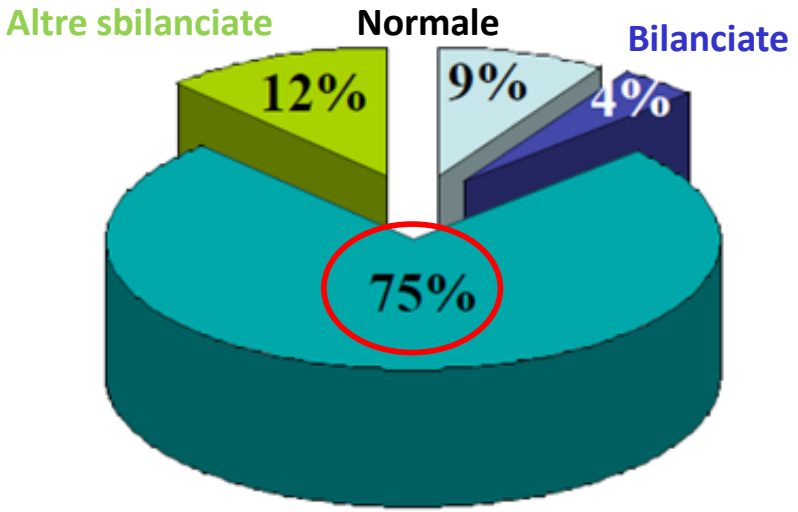
SMD de novo



Difetti dei cromosomi 5 e/o 7

Bilanciate
t(3;21)(q26;q21)
inv(3)(q21;q26)
t(11;16)(q23;p13.3)
t(1;3)(p36.3;q21.1)
t(2;11)(p21;q23)
t(6;9)(p23;q34)

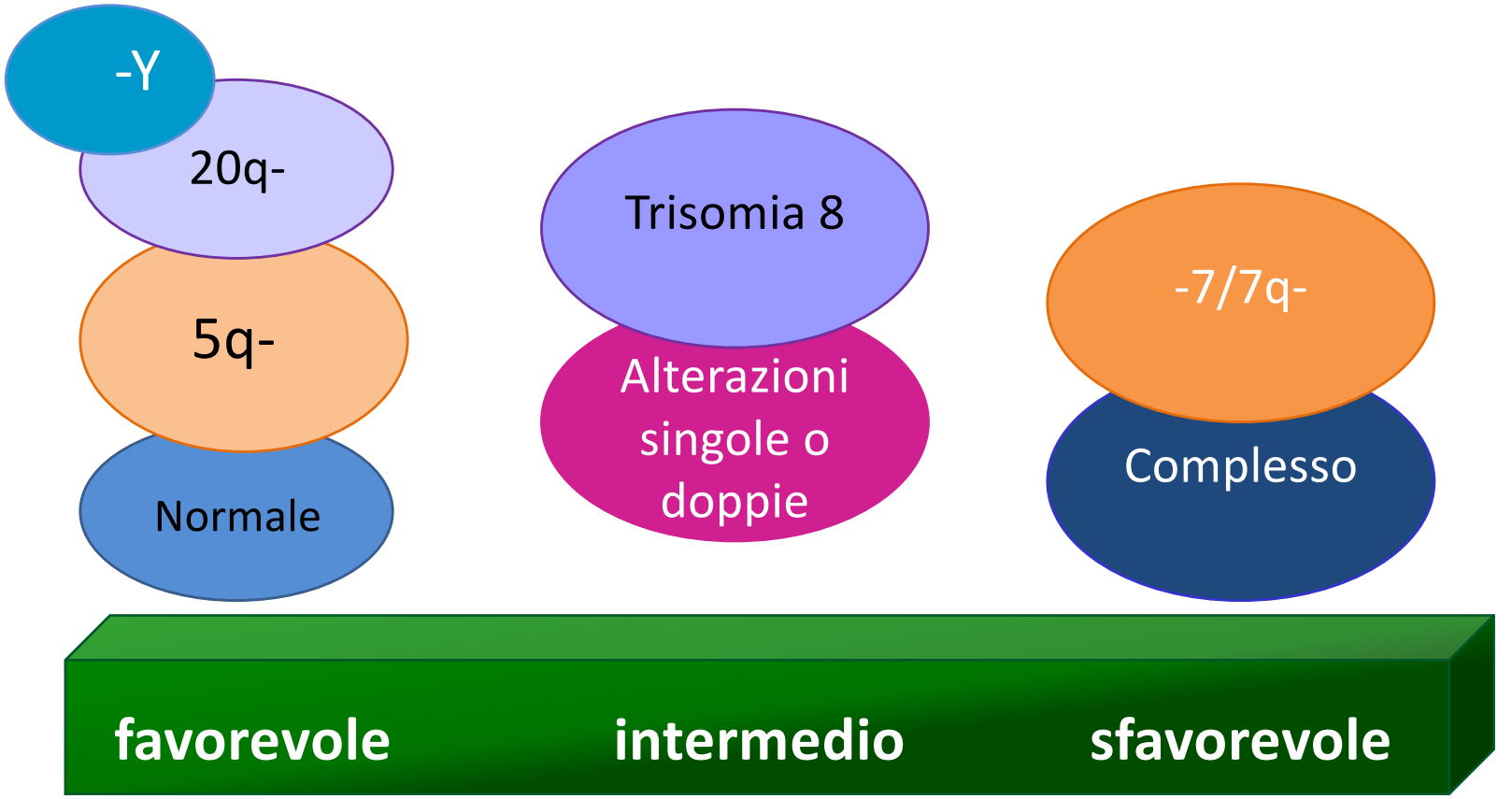
SMD secondary



Difetti dei cromosomi 5 e/o 7

Le Beau 2005

Alterazioni cromosomiche e rischio



WHO classification of Myelodysplastic Syndromes

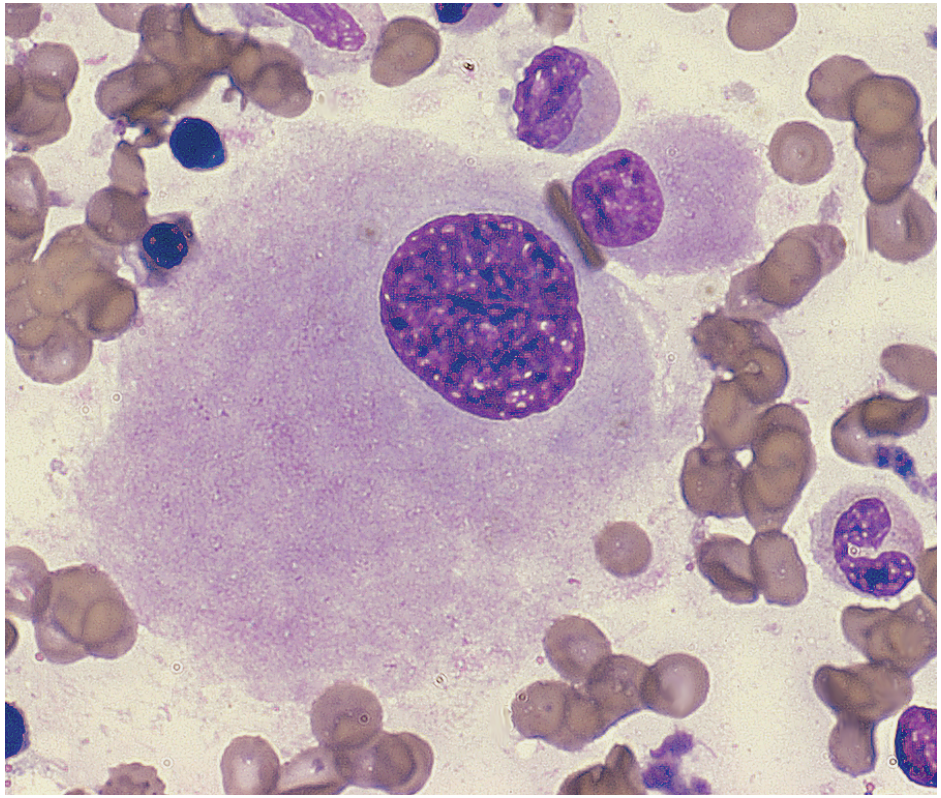
Disease	Blood findings	Bone marrow findings
Refractory cytopenia with unilineage dysplasia (RCUD):	Unicytopenia or bicytopenia* No or rare blasts (<1%)	Unilineage dysplasia: 10% of the cells in one myeloid lineage, <5% blasts, <15% of erythroid precursors are ring sideroblasts
Refractory anemia with ringed sideroblasts (RARS)	Anemia, no blasts.	Erythroid dysplasia only, < 5% blasts, ≥15% ringed sideroblasts.
Refractory cytopenia with multilineage dysplasia (RCMD)	Cytopenia(s), no or rare blasts (<1%), no Auer roads, $<1x10^9$/L monocytes.	Dysplasia in 10% of the cells in 2 myeloid lineages (neutrophil and/or erythroid precursors and/or megakaryocytes), <5% blasts in marrow No Auer rods, ±15% ring sideroblasts
Refractory anemia with excess blasts-1 (RAEB-1)	Cytopenia(s), <5% blasts, no Auer roads, $<1x\ 10^9$/L monocytes.	Unilineage or multilineage dysplasia, 5-9% blasts, no Auer roads.
Refractory anemia with excess blasts-2 (RAEB-2)	Cytopenia(s), 5-19% blasts, Auer roads ±, $<1x10^9$/L monocytes.	Unilineage or multilineage dysplasia, 10-19% blasts, Auer roads ±.
Myelodysplastic syndrome, unclassified (MDS-U)	Cytopenias, <1% blasts, no Auer roads.	Unequivocal dysplasia in <10% of cells in one or more myeloid lineages when accompanied by a cytogenetic abnormality considered as presumptive evidence for a diagnosis of MDS, <5% blasts
MDS associated with isolated del(5q)	Anemia, normal or increased platelet count, no or rare blasts (<1%)	Normal to increased megakaryocytes with hypolobated nuclei, <5% blasts, no Auer roads, isolated del(5q)

Blood 2008;114:937-51

Distinct haematological disorder with deletion of long arm of No. 5 chromosome

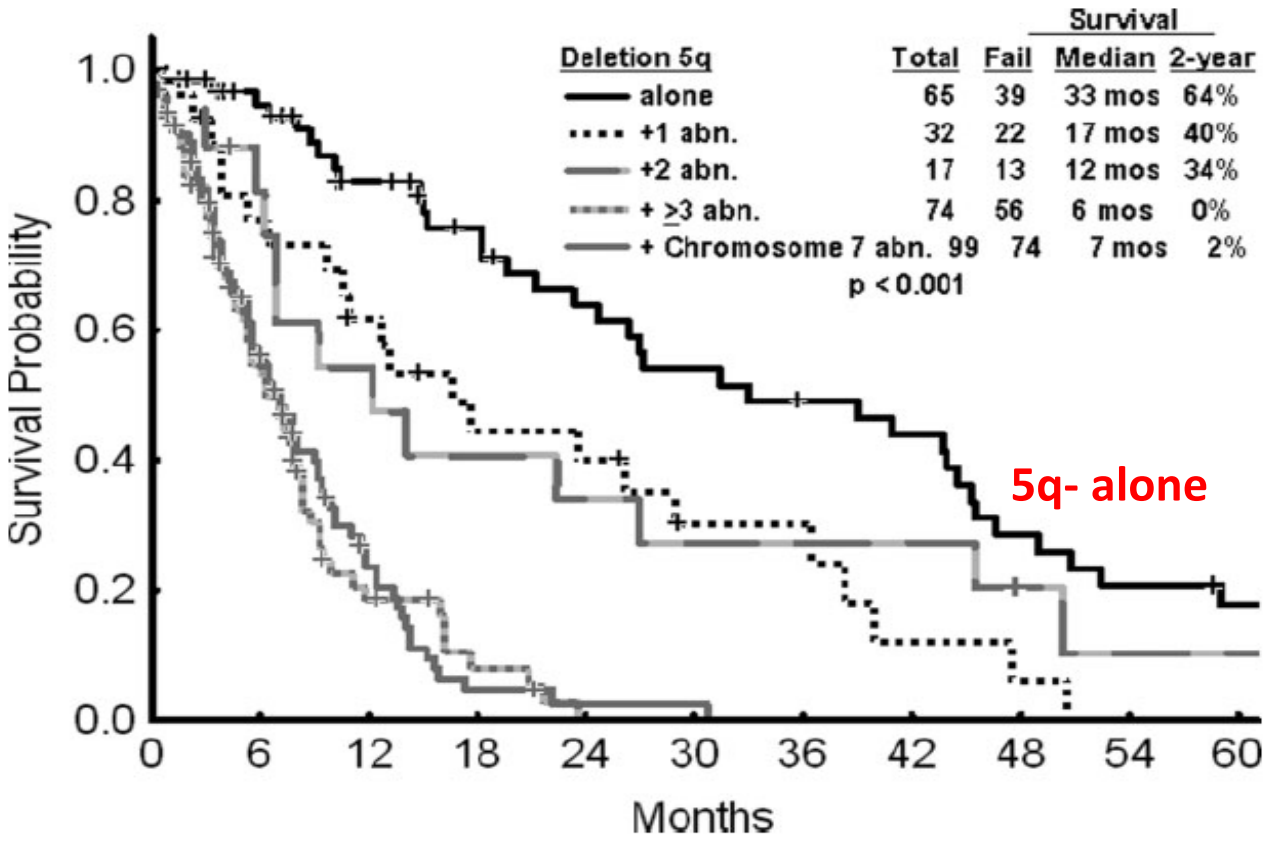
van Den Berghe, *Nature*, **251**, 437-438 (1974)

- Female preponderance
- 5q- sole karyotypic abnormality
- Macrocytic anemia (MCV>100 fL)
- High platelet count
- Increased megakaryocytes with monolobulated nuclei
- Prolonged survival



Delezione 5q e prognosi

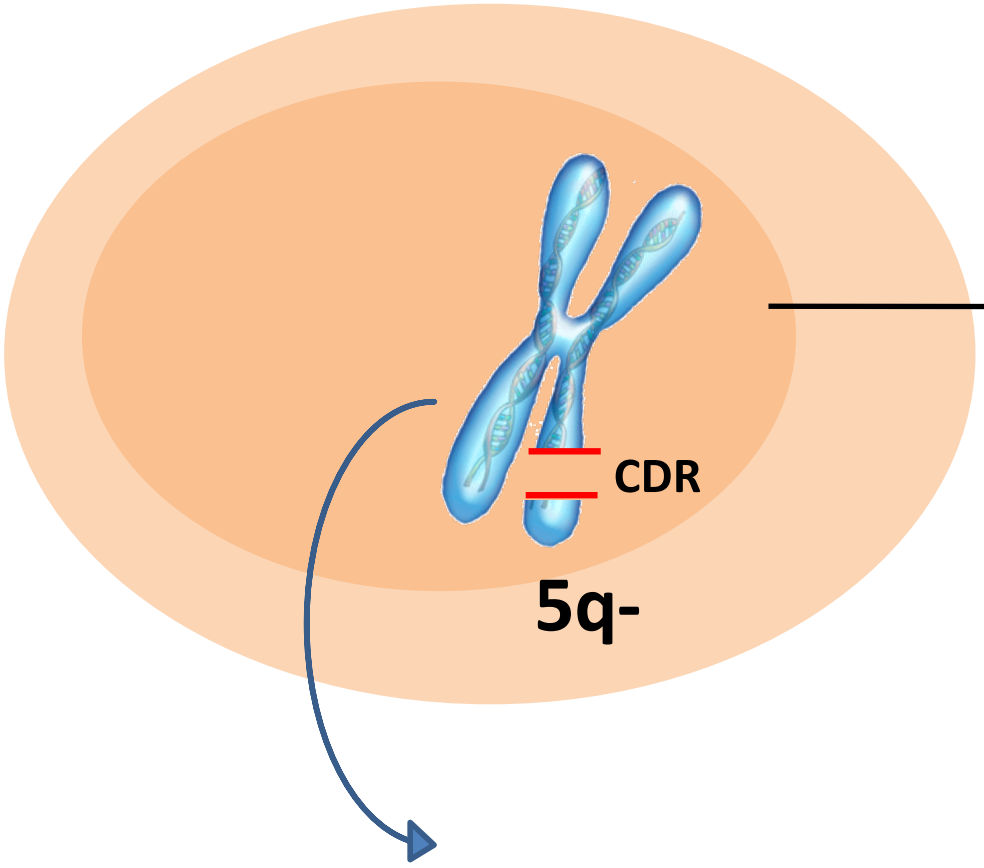
La **prognosi** delle delezioni 5q è generalmente **favorevole** a meno che tali delezioni non si verifichino contemporaneamente ad addizionali alterazioni citogenetiche



Kantarjan H, et al. Cancer 2009 5202-9

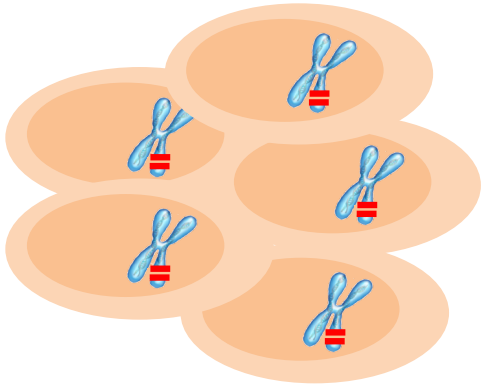
Aploinsufficienza

Genotipo-Fenotipo



APLOINSUFFICIENZA
per uno o più geni localizzati nella CDR → Riduzione del 50% della dose genica

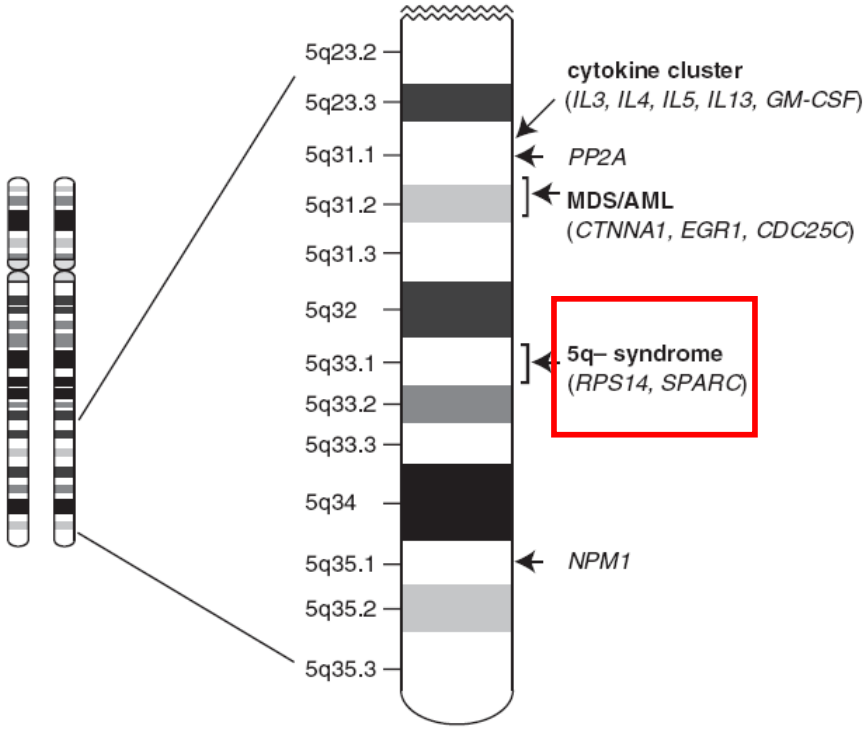
espansione midollare di un progenitore emopoietico con del(5q)



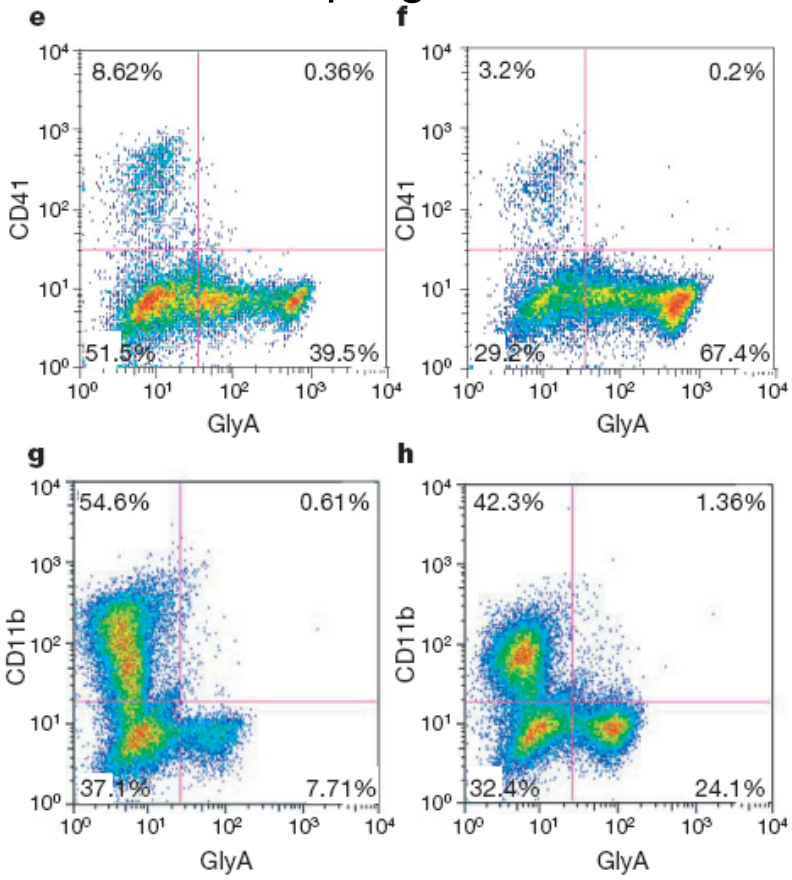
NO delezione biallelica o una mutazione puntiforme a carico della copia allelica non deleta → **APLOINSUFFICIENZA**

Identification of RPS14 as a 5q- syndrome gene by RNA interference screen

CDR of 5q- syndrome



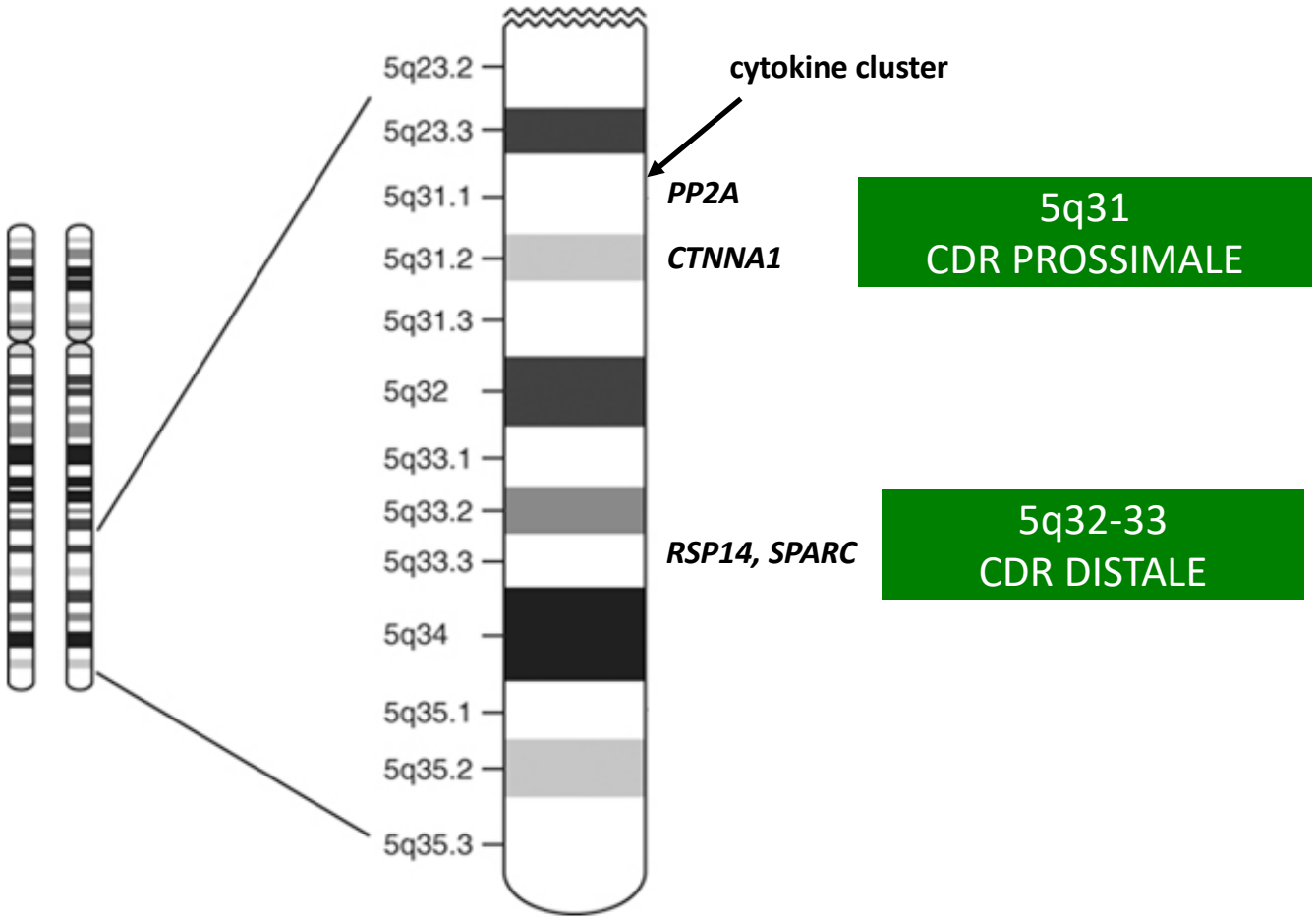
Normal progenitor cells



Blood. 2002;99:4638-4641

Nature. 2008;451(7176):335-339

Definizione della CDR

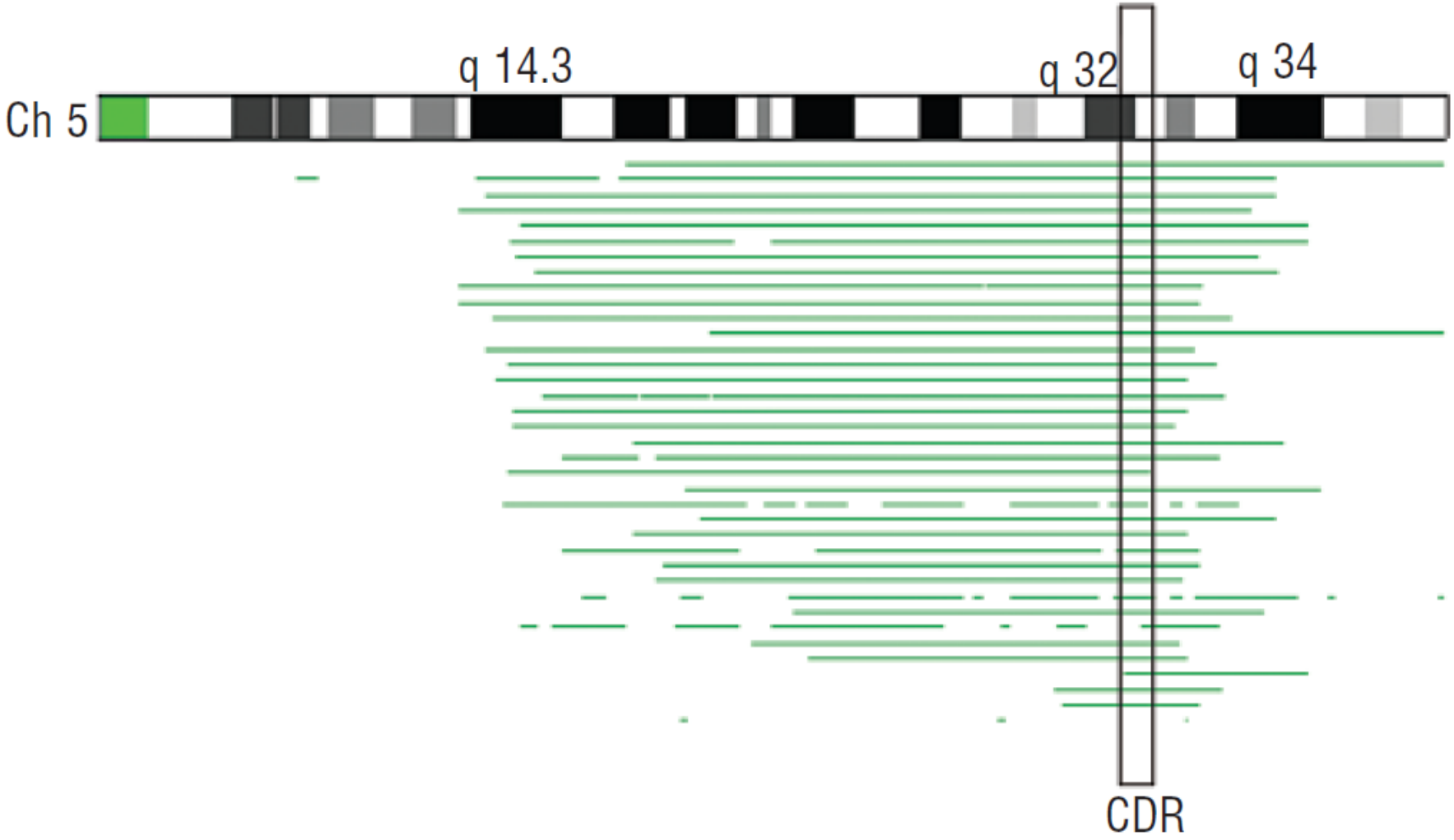


CDR: Common Deleted Region

Ebert BL, Leukemia 2009; 23: 1252-1256

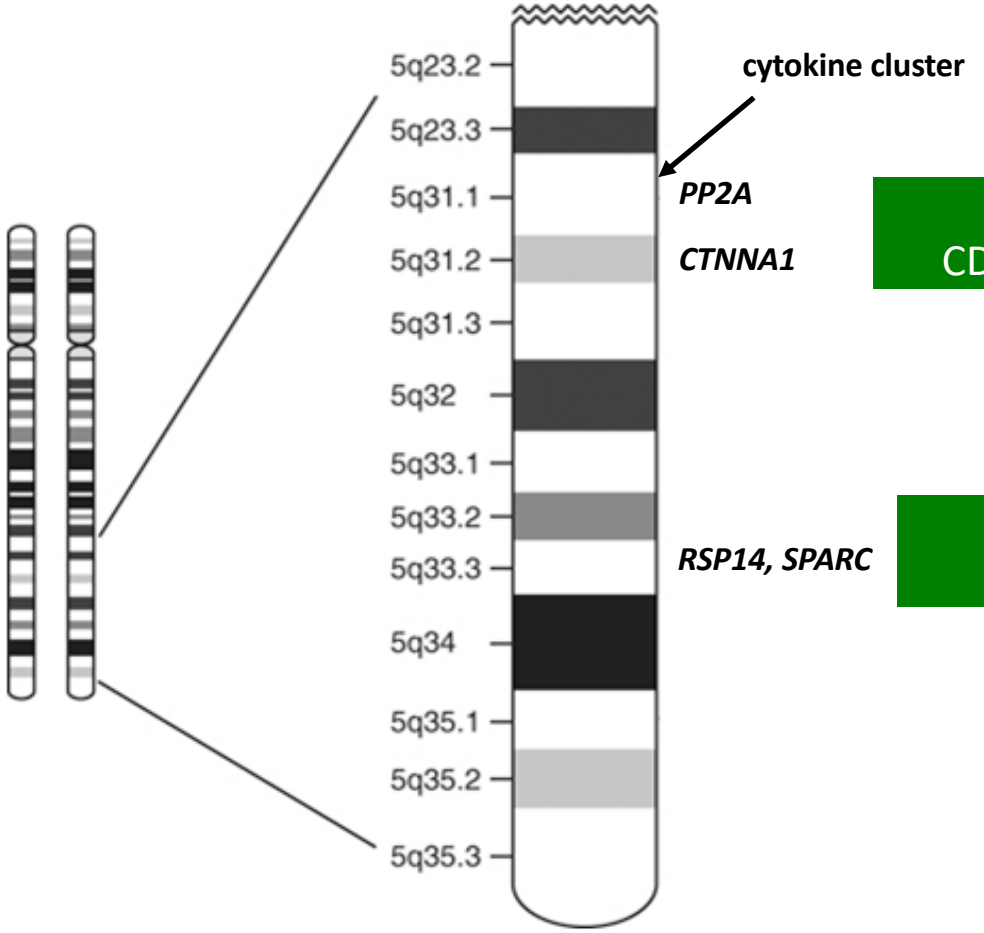
Definizione della CDR mediante SNP array

La tecnologia degli array basati sui polimorfismi del singolo nucleotide (**SNP array**) fornisce un nuovo potente strumento per caratterizzare le alterazioni genomiche e definire le minime regioni comuni di delezione.



Wang L Haematologica 2008

Definizione della CDR



5q31
CDR PROSSIMALE

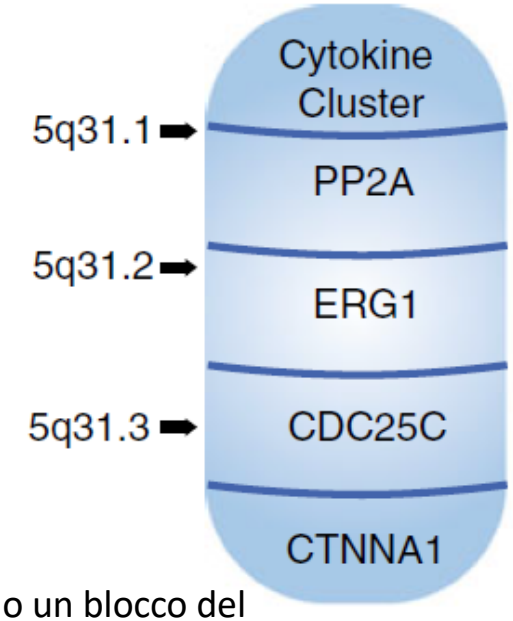
SMD ad alto rischio o LAM

5q32-33
CDR DISTALE

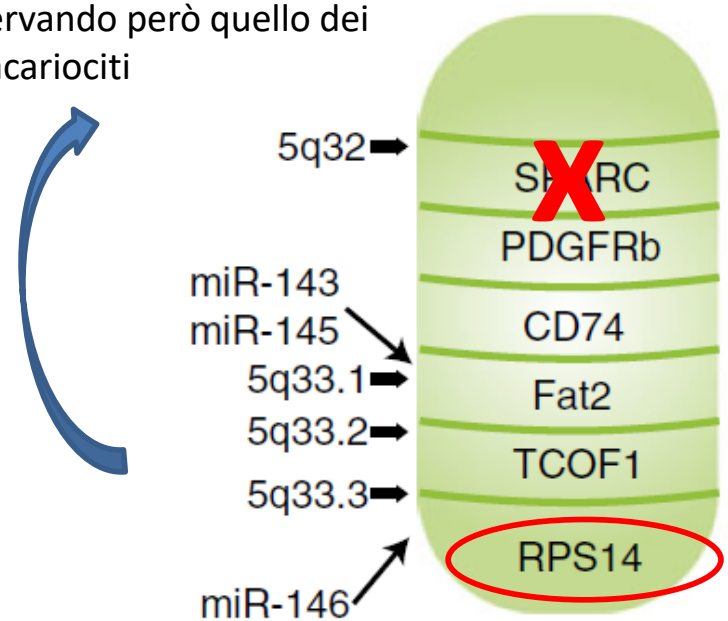
Sindrome 5q-

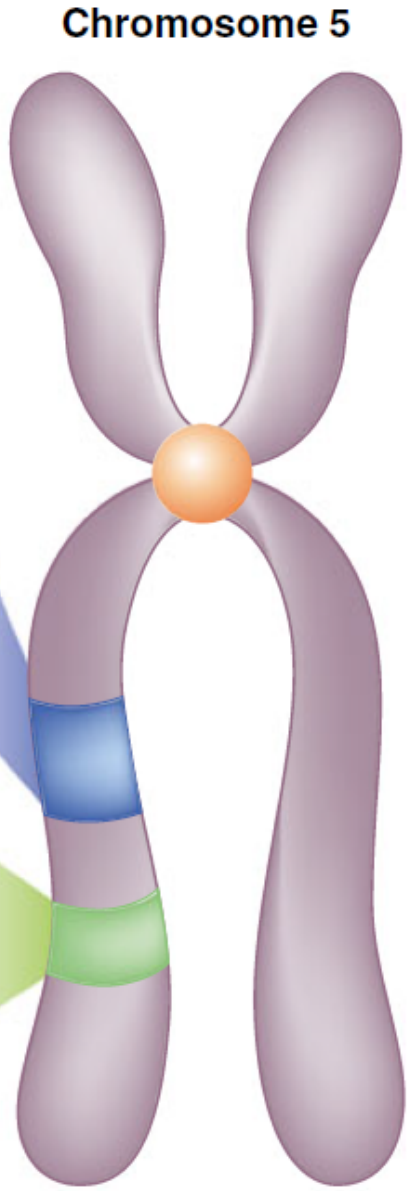
Ebert BL, Leukemia 2009; 23: 1252-1256

Identificazione di geni candidati



Geni che provocano un blocco del differenziamento in senso eritroide preservando però quello dei megacariociti





Identificazione di geni candidati

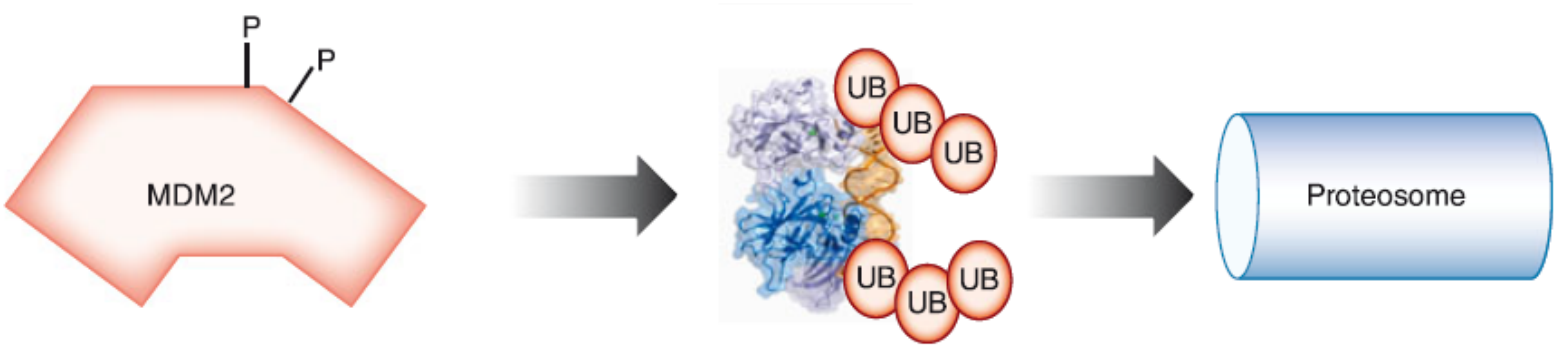
Interferenza con RNA (**RNAi**) su 40 geni localizzati nella CDR in 5q32-5q33 in colture di cellule CD34+ normali in condizioni favorenti la differenziazione in senso eritroide o megacariocitario

Padron E Curr Treat Options in Oncol. 2011
Ebert BL Nature 2008

Aploinsufficienza ribosomiale

Il **difetto funzionale** delle proteine ribosomali nelle cellule staminali emopoietiche causa: 1) apoptosi, 2) una riduzione della sintesi di emoglobina e 3) alterazione della trascrizione

1)

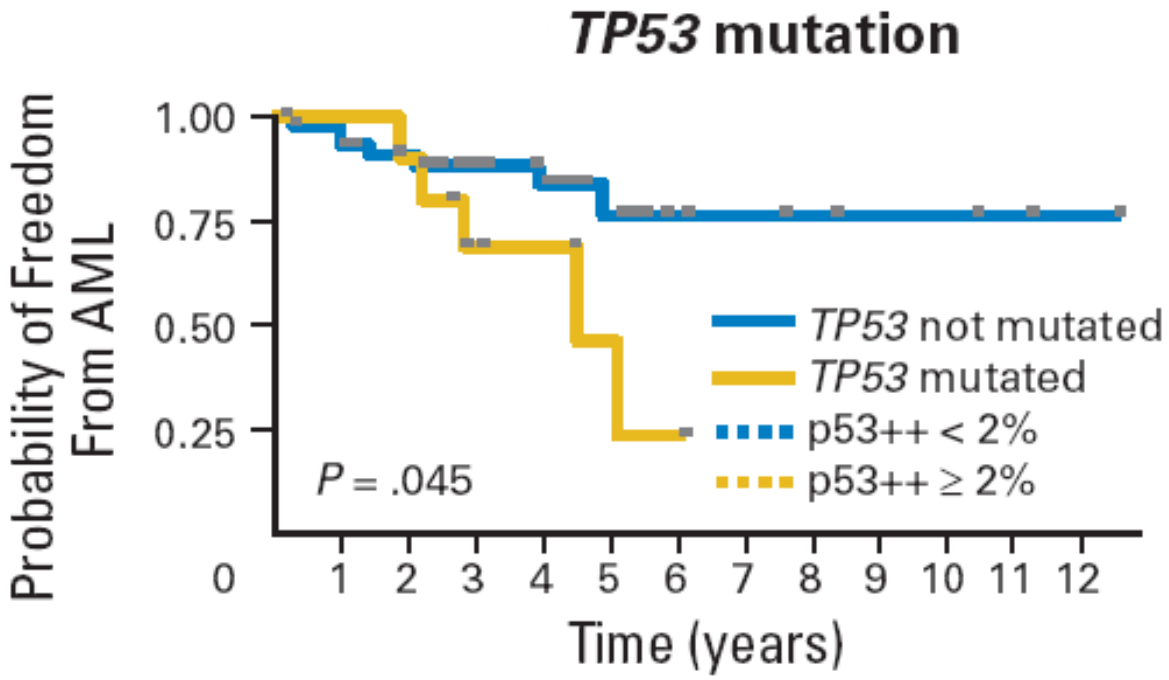


p53

APOPTOSI

Padron E Curr Treat Options in Oncol. 2011

TP53 Mutations in Low-Risk Myelodysplastic Syndromes With del(5q) Predict Disease Progression



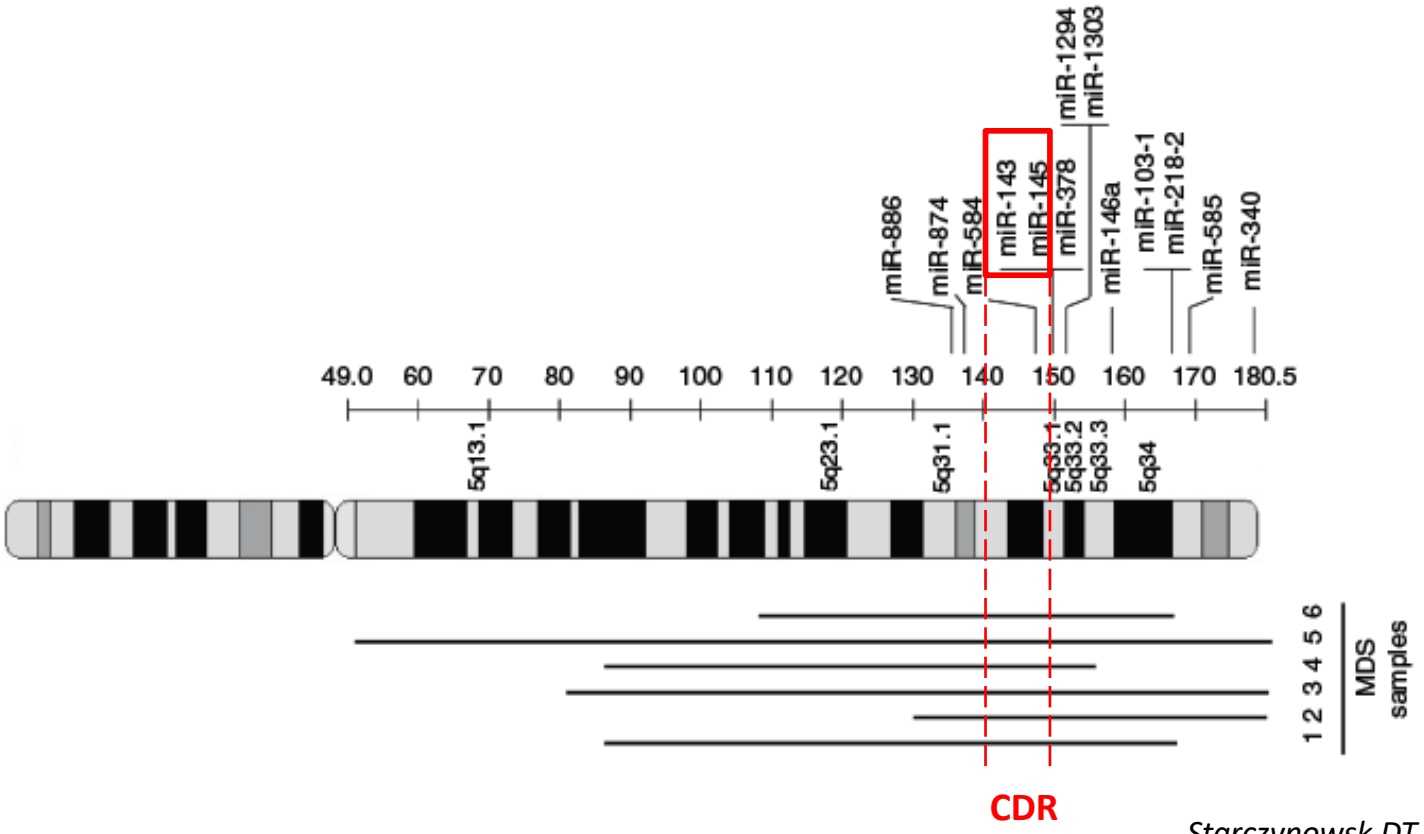
JCO 2011;29:1971-9

MicroRNAs in 5q- SMD

nature medicine

Identification of miR-145 and miR-146a as mediators of the 5q– syndrome phenotype

Daniel T Starczynowski[1,2], Florian Kuchenbauer[1], Bob Argiropoulos[1], Sandy Sung[1], Ryan Morin[1], Andrew Muranyi[1], Martin Hirst[1], Donna Hogge[1], Marco Marra[1], Richard A Wells[3], Rena Buckstein[3], Wan Lam[1,2], R Keith Humphries[1,4] & Aly Karsan[1,2]



Down-espressione di miR-143 e miR-145 nei pazienti con 5q-SMD

Starczynowsk DT, Nature Medicine 16, 49 - 58 (2010)

Lenalidomide in 5q- SMD

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Lenalidomide in the Myelodysplastic Syndrome with Chromosome 5q Deletion

Alan List, M.D., Gordon Dewald, Ph.D., John Bennett, M.D., Aristotle Giagounidis, M.D., Azra Raza, M.D., Eric Feldman, M.D., Bayard Powell, M.D., Peter Greenberg, M.D., Deborah Thomas, M.D., Richard Stone, M.D., Craig Reeder, M.D., Kenton Wride, M.S., John Patin, M.S., Michele Schmidt, R.N., Jerome Zeldis, M.D., and Robert Knight, M.D., for the Myelodysplastic Syndrome-003 Study Investigators*

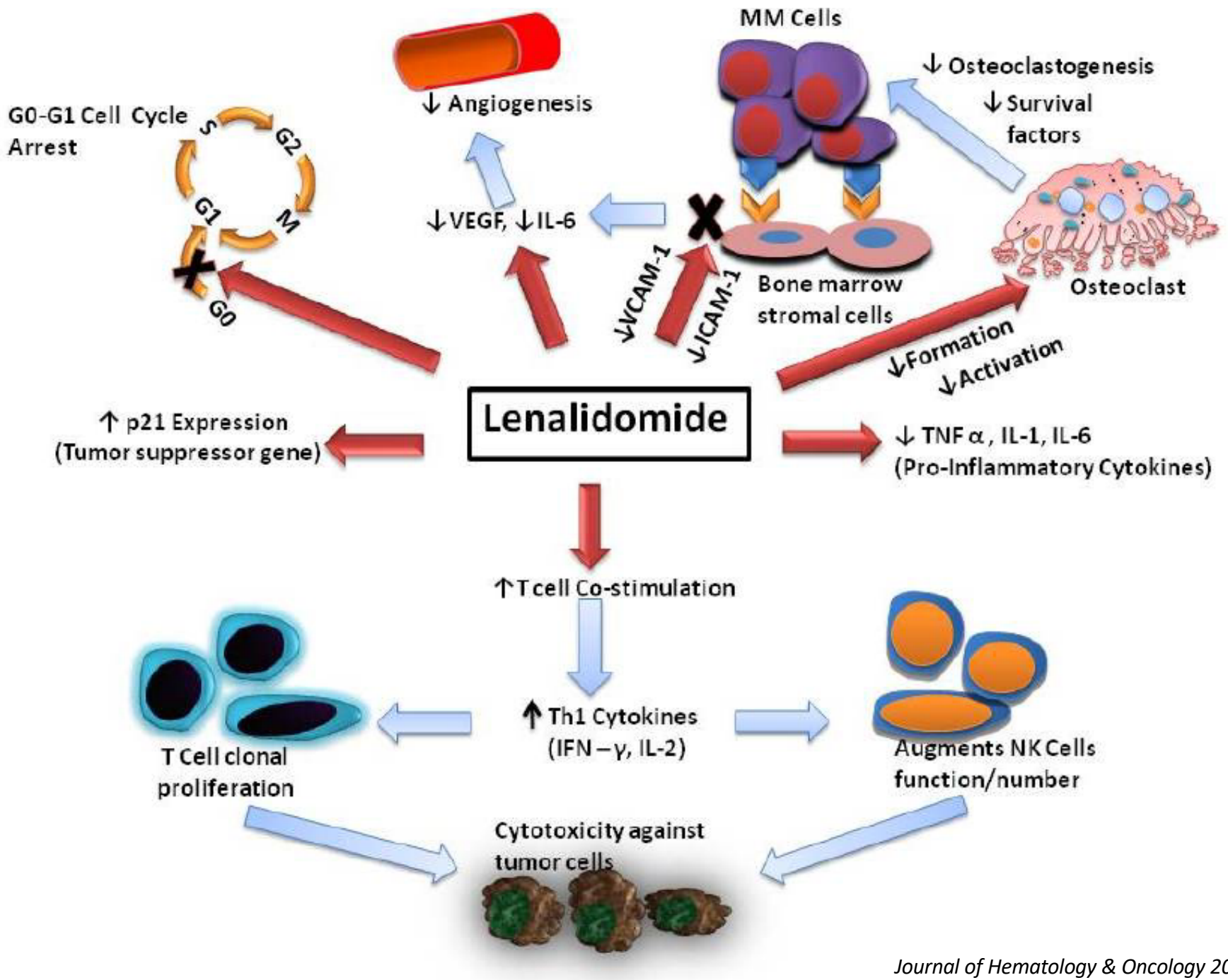
La lenalidomide è in grado di indurre la remissione citogenetica (50%) e di eliminare la dipendenza dalle trasfusioni (83%) nei pazienti con SMD e del(5q)

Table 3. Frequency of Cytogenetic Response According to Karyotype Complexity.

Complexity	Patients Who Could Be Evaluated*	Cytogenetic Response	Complete Cytogenetic Remission
Isolated 5q deletion — no. (%)	64	49 (77)	29 (45)
5q deletion + 1 additional abnormality — no. (%)	15	10 (67)	6 (40)
Complex (≥3 abnormalities) — no. (%)	6	3 (50)	3 (50)
P value		0.27	0.93

New Engl J Med 2006; 355:1456-1465

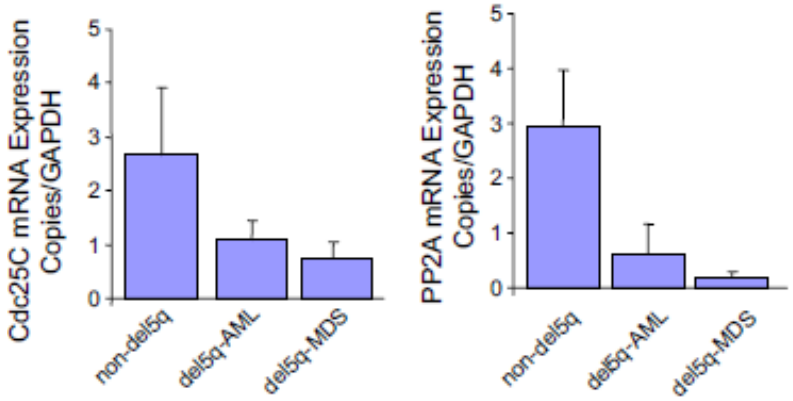
Meccanismo di azione della Lenalidomide in 5q- SMD

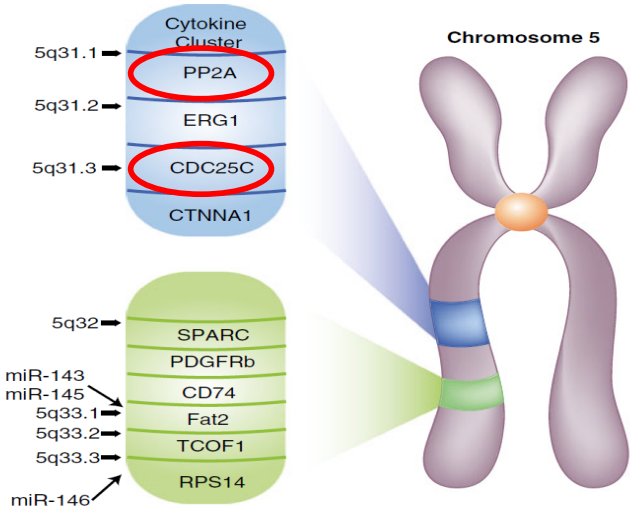


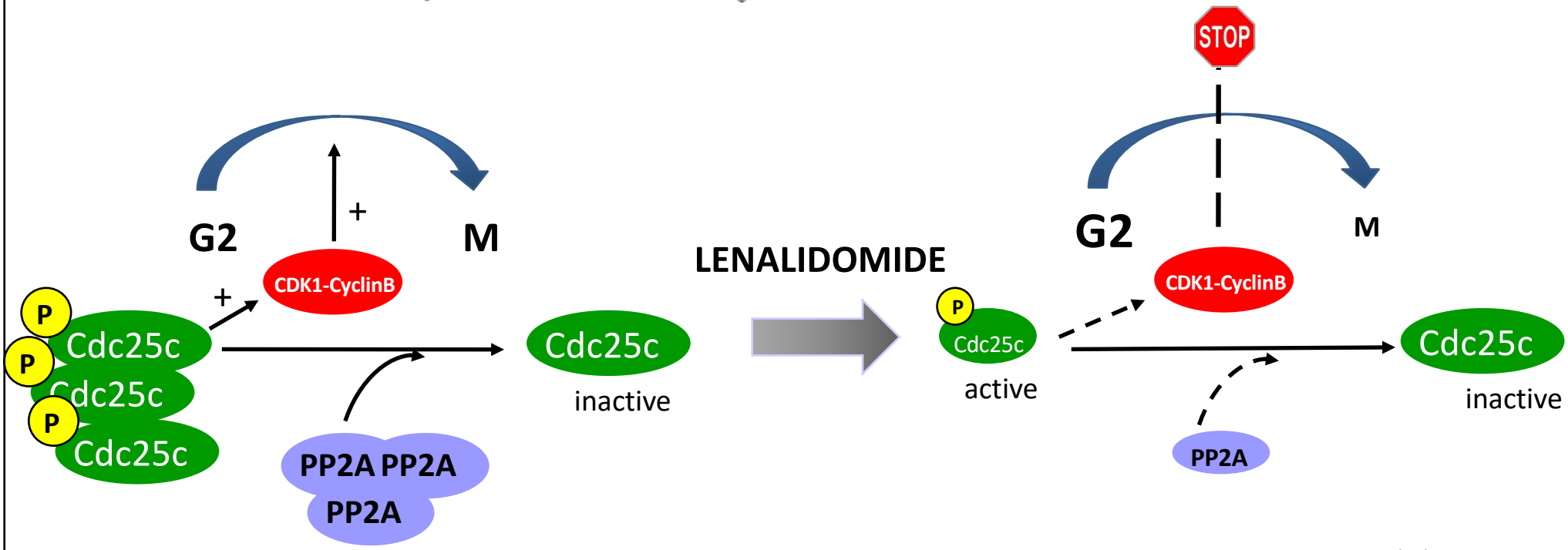
Journal of Hematology & Oncology 2009, 2:36

Meccanismo di azione della Lenalidomide in 5q- SMD

Riduzione del livello di espressione dei geni
CDC25C e PP2A nelle cellule con del(5q)





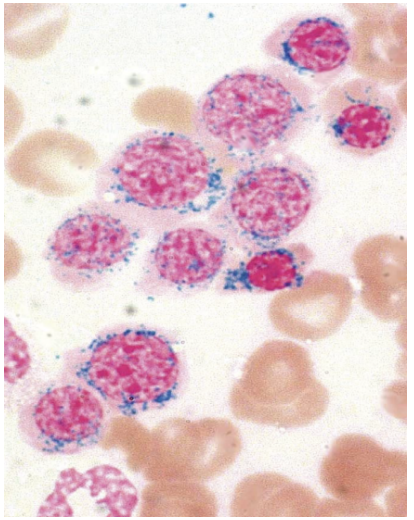
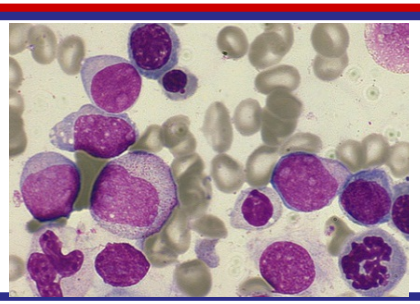


Wei S et al. PNAS 2009

2012 Molecular classification of MDS

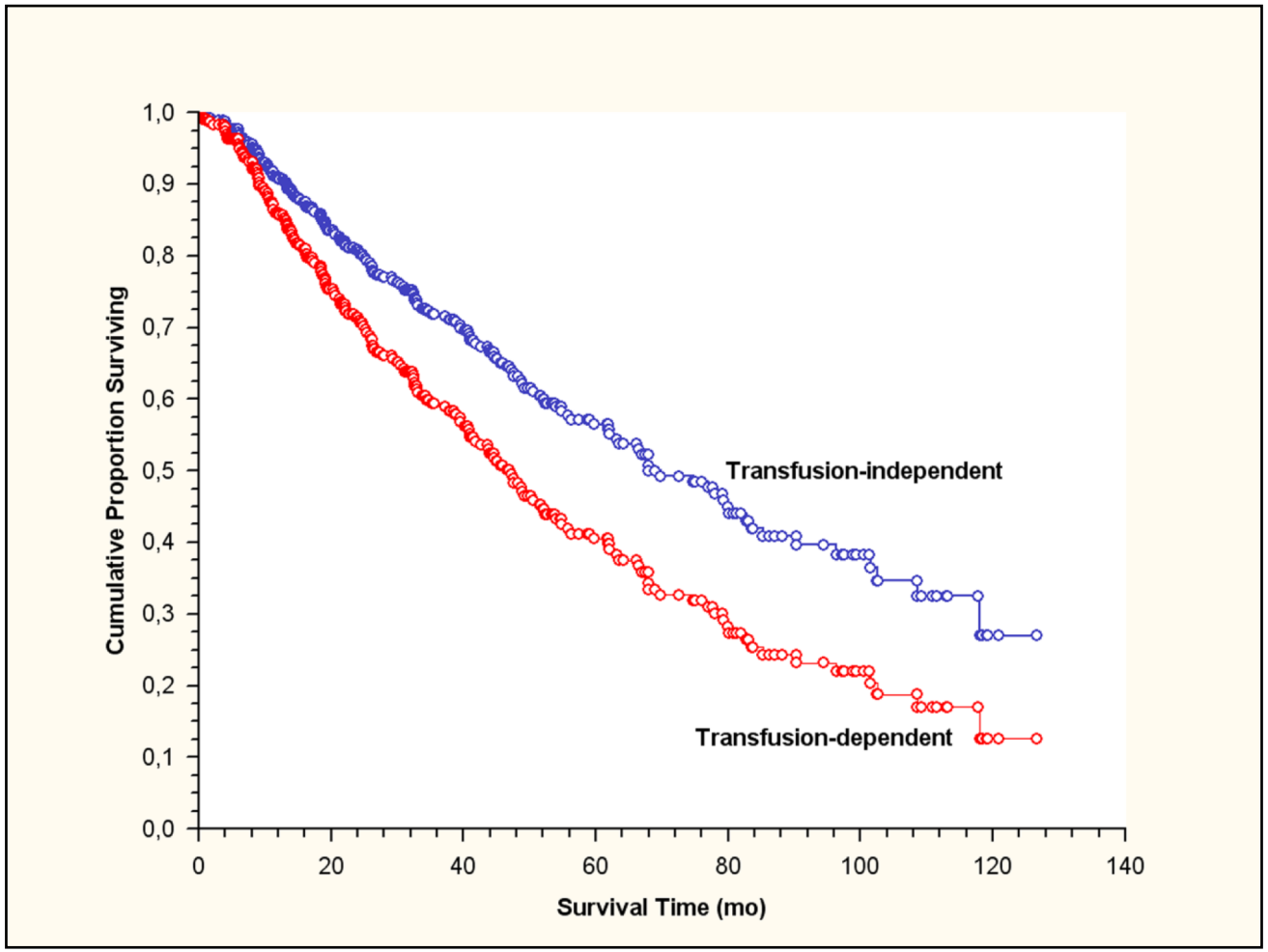
Disease	Molecular findings	Bone marrow findings
Refractory cytopenia with unilineage dysplasia (RCUD)		Erythroid dysplasia only, < 5% blasts, <15% ringed sideroblasts.
Refractory anemia with ringed sideroblasts (RARS)		Erythroid dysplasia only, < 5% blasts, ≥15% ringed sideroblasts.
MDS with isolated del(5q)	**RPS14, miR145, miR146 TP53 (leukemic evolution)**	Normal to increased megakaryocytes with hypolobated nuclei, <5% blasts, no Auer roads, isolated del(5q)
Refractory cytopenia with multilineage dysplasia (RCMD-RS)		Dysplasia in ≥ 10% of cells in 2 or more myeloid cell lines, < 5% blasts, no Auer roads, <15% ringed sideroblasts.
Refractory anemia with excess blasts-1 (RAEB-1)		Unilineage or multilineage dysplasia, 5% to 9% blasts, no Auer roads.
Refractory anemia with excess blasts-2 (RAEB-2)		Unilineage or multilineage dysplasia, 10% to 19% blasts, occasional Auer roads.
MDS with Marrow Fibrosis		Increased marrow cellularity, multilineage dysplasia, bone marrow fibrosis, presence of clusters of CD34+ cells.

WHO classification of Myelodysplastic Syndromes

Disease	Blood findings	Bone marrow findings
Refractory cytopenia with unilineage dysplasia (RCUD):	Unicytopenia or bicytopenia* No or rare blasts (<1%)	
Refractory anemia with ringed sideroblasts (RARS)	Anemia, no blasts.	
Refractory cytopenia with multilineage dysplasia (RCMD)	Cytopenia(s), no or rare blasts (<1%), no Auer roads, <1x10^9/L monocytes.	
Refractory anemia with excess blasts-1 (RAEB-1)	Cytopenia(s), <5% blasts, no Auer roads, <1x 10^9/L monocytes.	
Refractory anemia with excess blasts-2 (RAEB-2)	Cytopenia(s), 5-19% blasts, Auer roads ±, <1x10^9/L monocytes.	
Myelodysplastic syndrome, unclassified (MDS-U)	Cytopenias, <1% blasts, no Auer roads.	Unequivocal dysplasia in <10% of cells in one or more myeloid lineages when accompanied by a cytogenetic abnormality considered as presumptive evidence for a diagnosis of MDS, <5% blasts
MDS associated with isolated del(5q)	Anemia, normal or increased platelet count, no or rare blasts (<1%)	Normal to increased megakaryocytes with hypolobated nuclei, <5% blasts, no Auer roads, isolated del(5q)

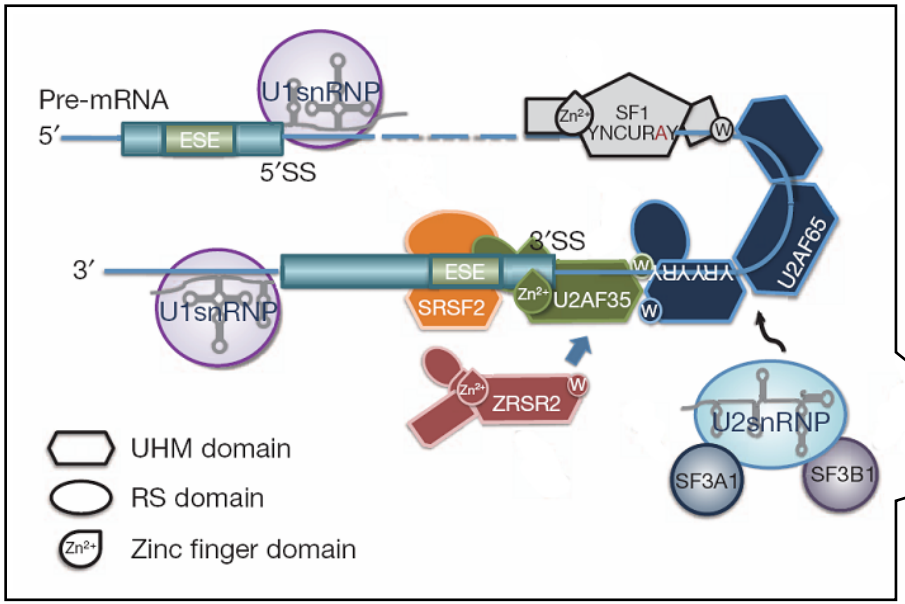
Blood 2008;114:937-51

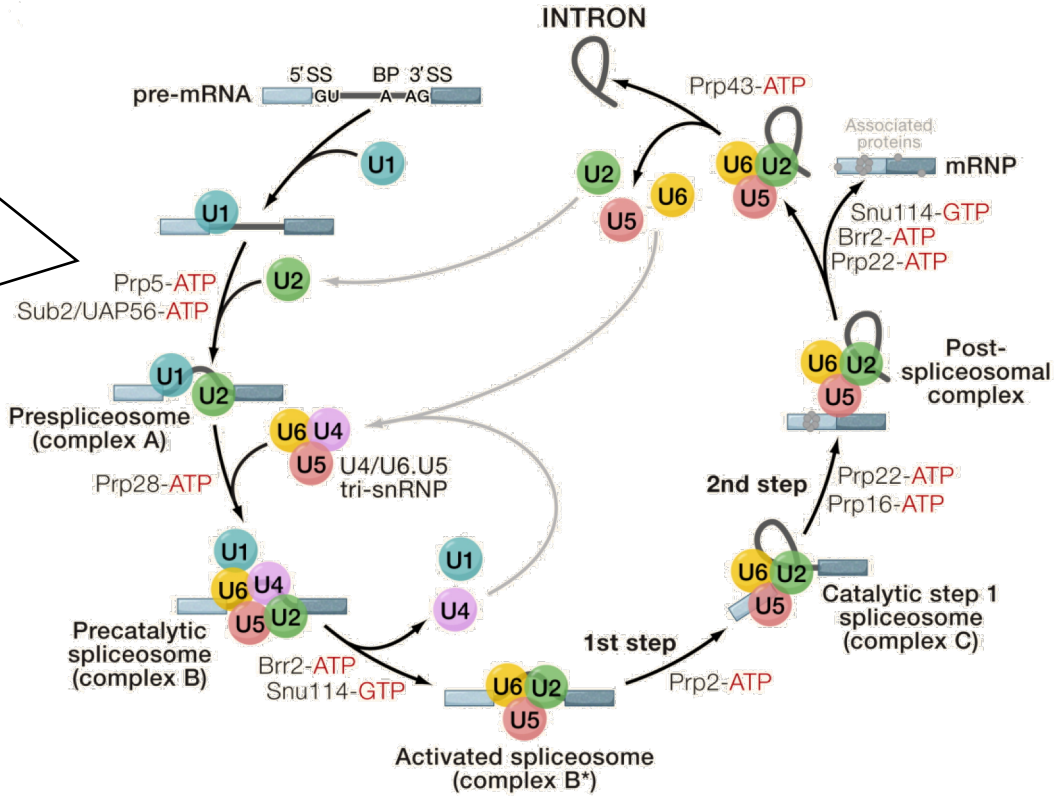
Survival of MDS patients according to transfusion-dependency



N Engl J Med 2005;352:536-8

RNA splicing machinery





Adapted from Cell 2009;136:701-18
and Nature 2011;478:64-9

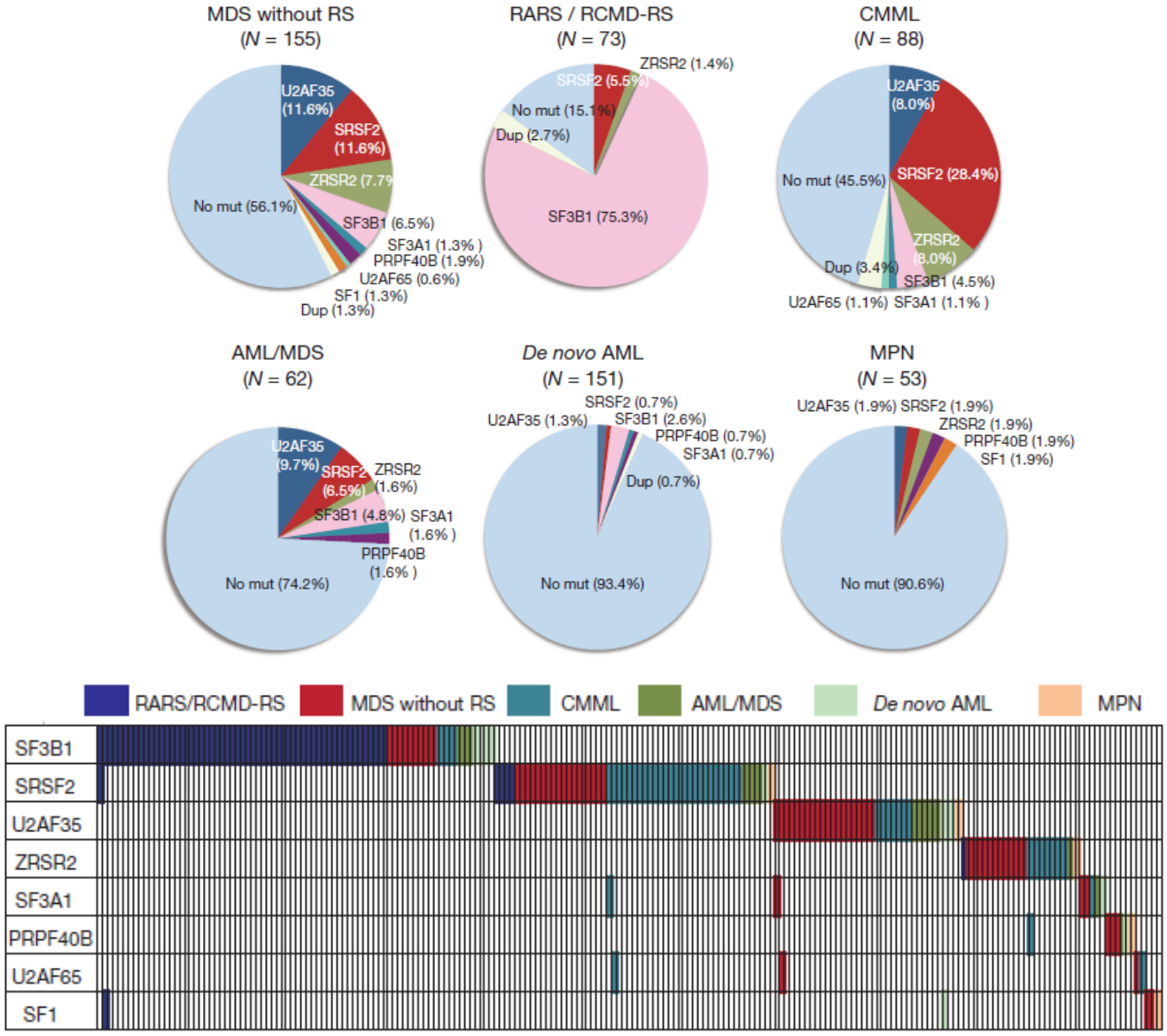
Somatic *SF3B1* Mutation in Myelodysplasia with Ring Sideroblasts

Tumor Type	Mutations	
	no. of patients/total no.	*% (95% CI)*
Myelodysplastic syndromes	72/354	20 (16–25)
Refractory anemia	9/91	10 (5–18)
Refractory anemia with ring sideroblasts	40/59	68 (54–79)
Refractory cytopenia with multilineage dysplasia	3/53	6 (1–16)
Refractory cytopenia with multilineage dysplasia and ring sideroblasts	13/23	57 (35–77)
Refractory anemia with excess blasts	6/110	6 (2–12)
Other subtypes	1/18	6 (0–27)

65%

N Engl J Med 2011; 365:1384-1395

Frequent pathway mutations of splicing machinery in myelodysplasia

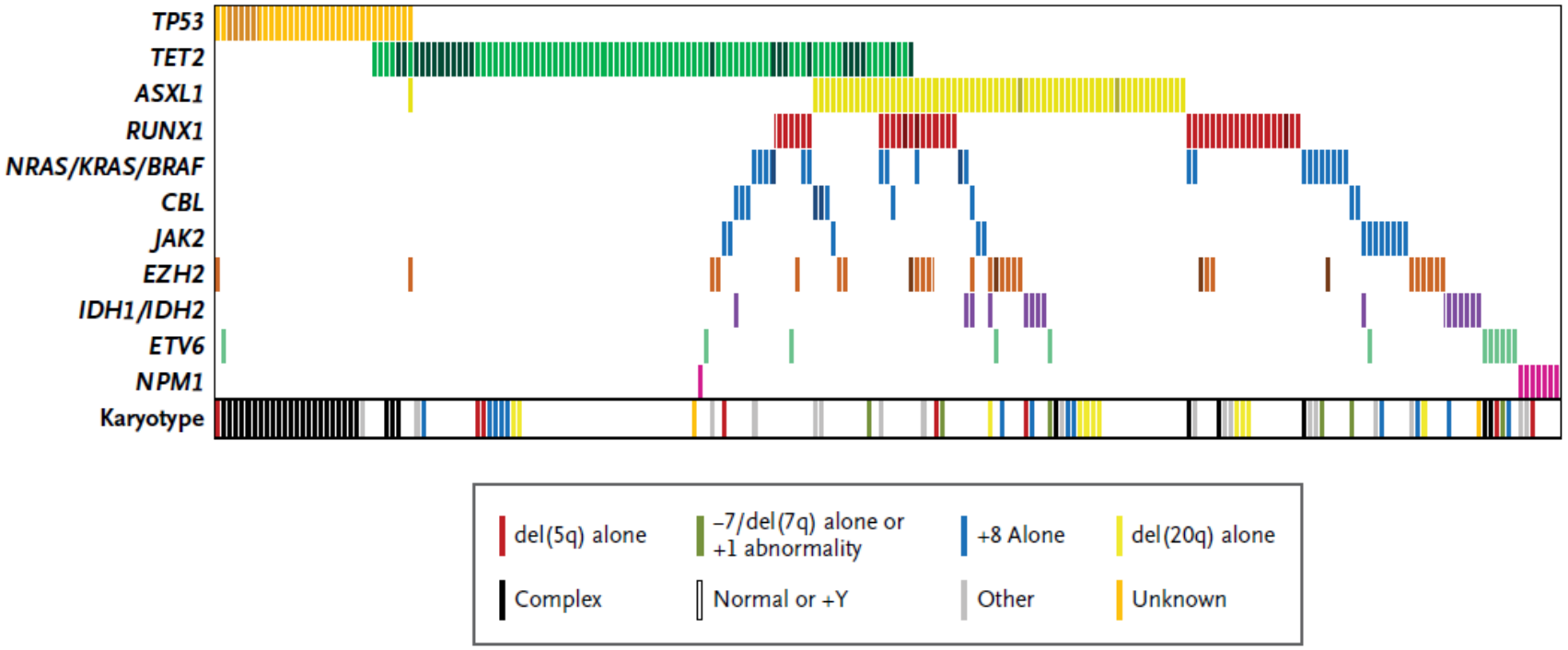


Nature. 2011 Sep 11;478(7367):64-9

2012 Molecular classification of MDS

Disease	Molecular findings	Bone marrow findings
Refractory cytopenia with unilineage dysplasia (RCUD)	**U2AF35**	Erythroid dysplasia only, < 5% blasts, <15% ringed sideroblasts.
Refractory anemia with ringed sideroblasts (RARS)	**SF3B1**	Erythroid dysplasia only, < 5% blasts, ≥15% ringed sideroblasts.
MDS with isolated del(5q)	**RPS14, miR145, miR146 TP53 (leukemic evolution)**	Normal to increased megakaryocytes with hypolobated nuclei, <5% blasts, no Auer roads, isolated del(5q)
Refractory cytopenia with multilineage dysplasia (RCMD)	**SRSF2, U2AF35**	Dysplasia in ≥ 10% of cells in 2 or more myeloid cell lines, < 5% blasts, no Auer roads, <15% ringed sideroblasts.
Refractory anemia with excess blasts-1 (RAEB-1)		Unilineage or multilineage dysplasia, 5% to 9% blasts, no Auer roads.
Refractory anemia with excess blasts-2 (RAEB-2)		Unilineage or multilineage dysplasia, 10% to 19% blasts, occasional Auer roads.
MDS with Marrow Fibrosis		Increased marrow cellularity, multilineage dysplasia, bone marrow fibrosis, presence of clusters of CD34+ cells.

Clinical Effect of Point Mutations in Myelodysplastic Syndromes



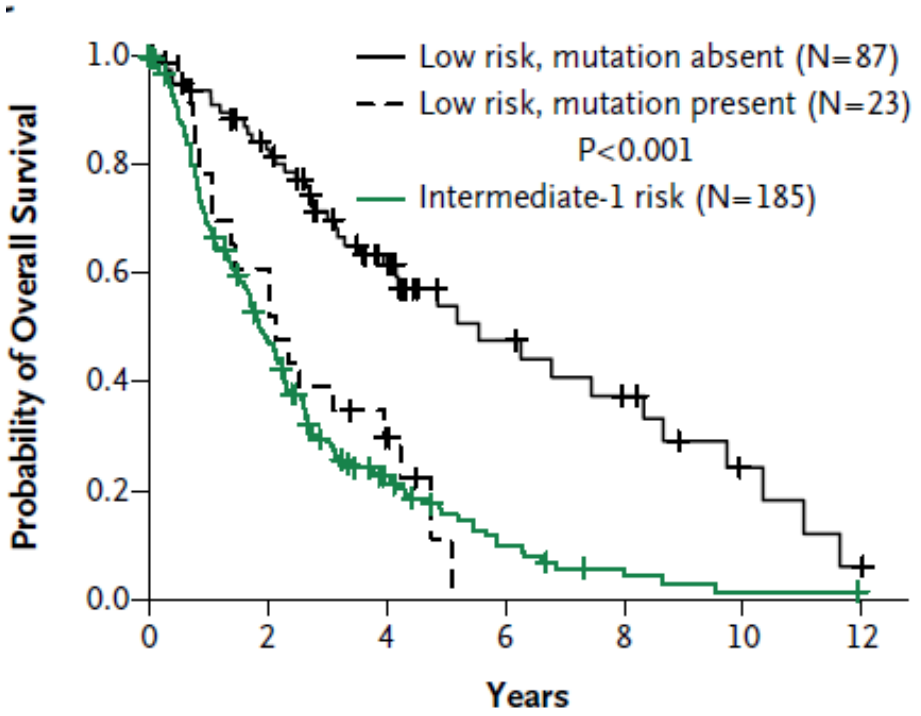
- By OncoMap screening, somatic mutations were identified in 18 genes.
- 50.9% of samples were found to carry at least one mutation
- Most frequently mutated genes were *TET2* (18%), *ASXL1* (14%), *RUNX1* (8%), and *TP53* (7%).

N Engl J Med 2011;364:2496-506

Clinical Effect of Point Mutations in Myelodysplastic Syndromes

Table 2. Hazard Ratios for Death in a Multivariable Model.*

Risk Factor	Hazard Ratio (95% CI)	P Value
Age ≥55 yr vs. <55 yr	1.81 (1.20–2.73)	0.004
IPSS risk group		
Intermediate-1 vs. low	2.29 (1.69–3.11)	<0.001
Intermediate-2 vs. low	3.45 (2.42–4.91)	<0.001
High vs. low	5.85 (3.63–9.40)	<0.001
Mutational status		
TP53 mutation present vs. absent	2.48 (1.60–3.84)	<0.001
EZH2 mutation present vs. absent	2.13 (1.36–3.33)	<0.001
ETV6 mutation present vs. absent	2.04 (1.08–3.86)	0.03
RUNX1 mutation present vs. absent	1.47 (1.01–2.15)	0.047
ASXL1 mutation present vs. absent	1.38 (1.00–1.89)	0.049

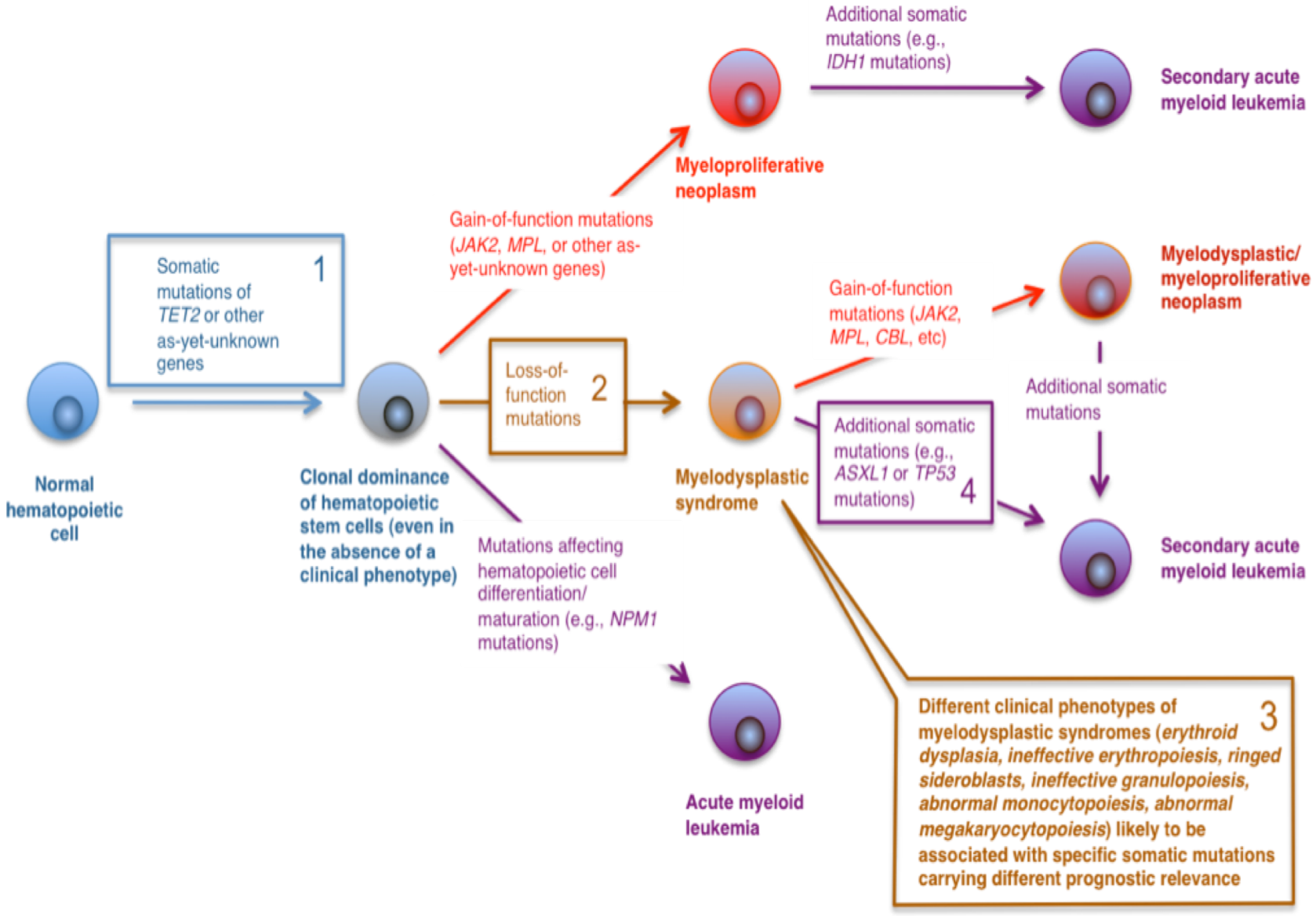


N Engl J Med 2011;364:2496-506

Molecular classification of MDS

Disease	Molecular findings	Bone marrow findings
Refractory cytopenia with unilineage dysplasia (RCUD)	**SRSF2, U2AF35**	Erythroid dysplasia only, < 5% blasts, <15% ringed sideroblasts.
Refractory anemia with ringed sideroblasts (RARS)	**SF3B1**	Erythroid dysplasia only, < 5% blasts, ≥15% ringed sideroblasts.
MDS with isolated del(5q)	**RPS14, miR145, miR146** **TP53 (leukemic evolution)**	Normal to increased megakaryocytes with hypolobated nuclei, <5% blasts, no Auer roads, isolated del(5q)
Refractory cytopenia with multilineage dysplasia (RCMD-RS)	**SF3B1**	Dysplasia in ≥ 10% of cells in 2 or more myeloid cell lines, < 5% blasts, no Auer roads, <15% ringed sideroblasts.
Refractory anemia with excess blasts-1 (RAEB-1)	**TP53** **ASXL1** **RUNX1** **EZH2** **ETV6**	Unilineage or multilineage dysplasia, 5% to 9% blasts, no Auer roads.
Refractory anemia with excess blasts-2 (RAEB-2)		Unilineage or multilineage dysplasia, 10% to 19% blasts, occasional Auer roads.

Molecular pathogenesis of Myeloid Neoplasms



Acknowledgments

Supported by: FP7, Harmony, IOR, European LeukemiaNet, AIL, AIRC, FIRB 2006, Fondazione del Monte di Bologna e Ravenna