FONDAZIONE ITALIANA SINDROMI MIELODISPLASTICHE

L'attuale approccio clinico al paziente con **Sindrome Mielodisplastica**

Aspetti immunologici nelle Mielodisplasie Ipoplastiche

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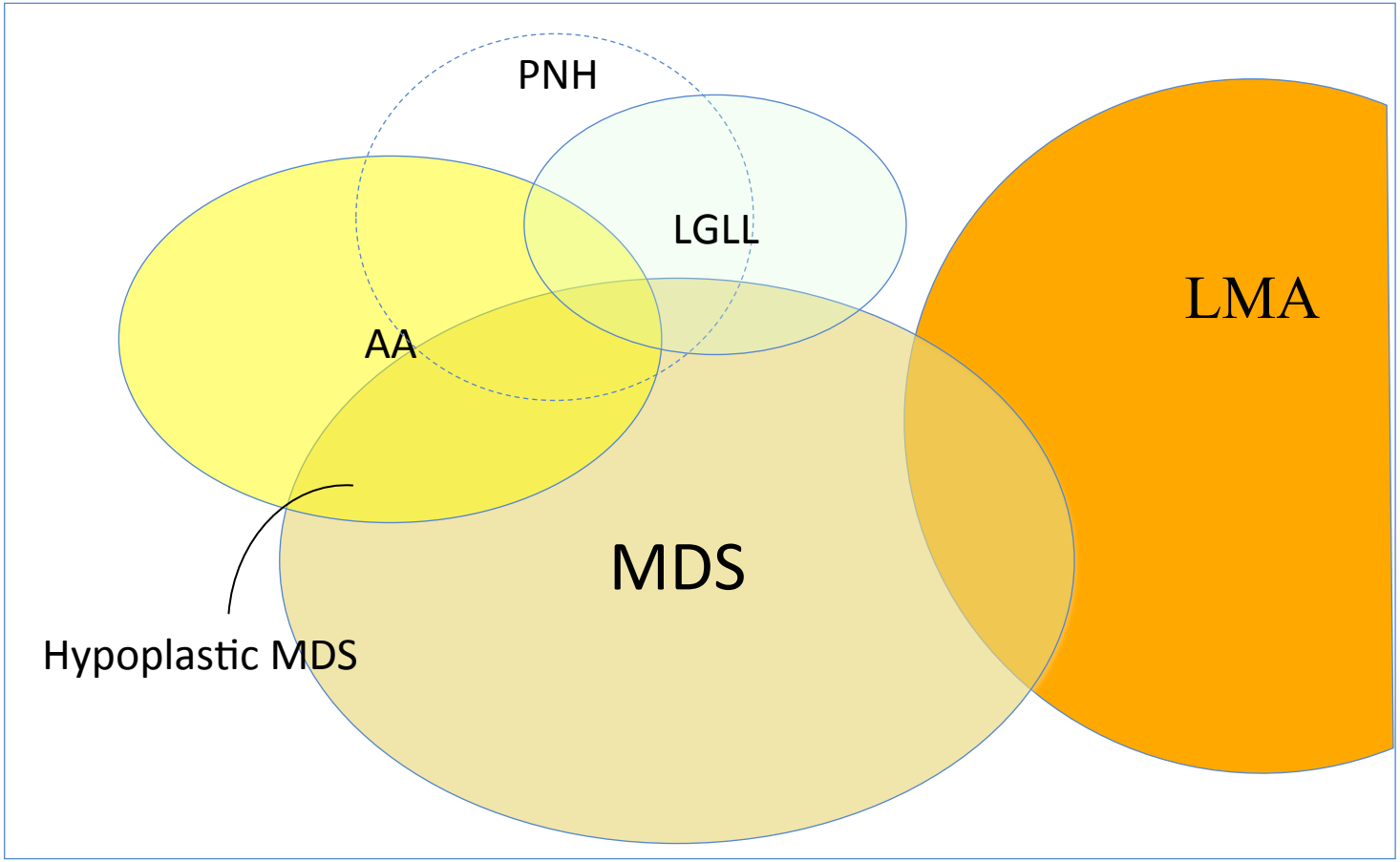
Department of Medicine
Hematology and Clinical Immunology

Bone marrow failure disorders

Diagnosis can be difficult due to overlapping between each entity



modified from Young NS, Ann Intern Med, 2002

Bone marrow failure disorders: common features among AA, hMDS and LGLL

- immune system involvement ("immune attack")
- stem cell (progenitors) as target


Che cos’è?
E’ un pugnale
E’ un serpente
E’ un albero
E’ una corda

Patient RA, 55 y

Analysis	Results	Reference	Units
WBC	2.2	3.5-10	x10e9/l
RBC	2.74	4.20-5.40	x10e12/l
HB	90	140-180	g/l
HT	0.28	0.36-0.46	l/l
MCV	95	79-95	fl
MCH	33	27-33.2	pg
MCHC	322	320-360	g/l
RDW	14.8	11.5-14.5	%
Platelets	30	150-450	x10e9/l
Reticulocyte	50	40-140	x10e9/l
Neutrophils	0.40	1.30-10.70	x10e9/l
Lymphocyte	1.304	0.900-3.300	x10e9/l
Monocyte	0.550	0.120-0.620	x10e9/l

Potrebbe trattarsi:

- AA? si
- MDS? si
- LGL? si
- Altro? si

Peripheral blood in AA

Anemia is usually present

-Hypo or aregenerative

- Low count of reticulocyte

-Macrocytosis is common

Careful examination of the blood film to exclude:

-dysplastic neutrophils

-abnormal platelets

-blasts and other abnormal cells, such as hairy cells, LGL

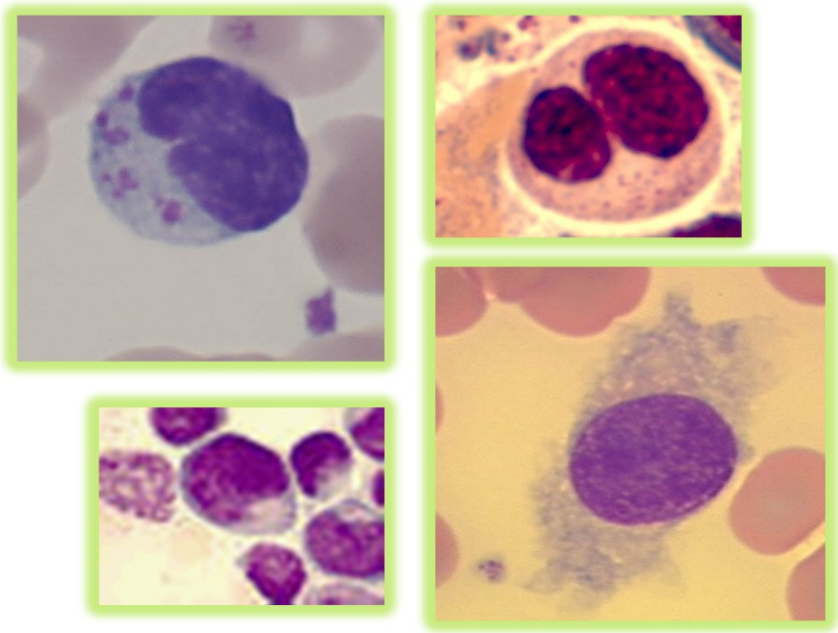
Neutropenia is frequent

Lymphocyte count is usually preserved

Monocytopenia

Thrombocytopenia

Early stages as isolated cytopenia (DD ITP)



Bone Marrow Examinations

Required:

- bone marrow aspirate
- trephine biopsy should be done

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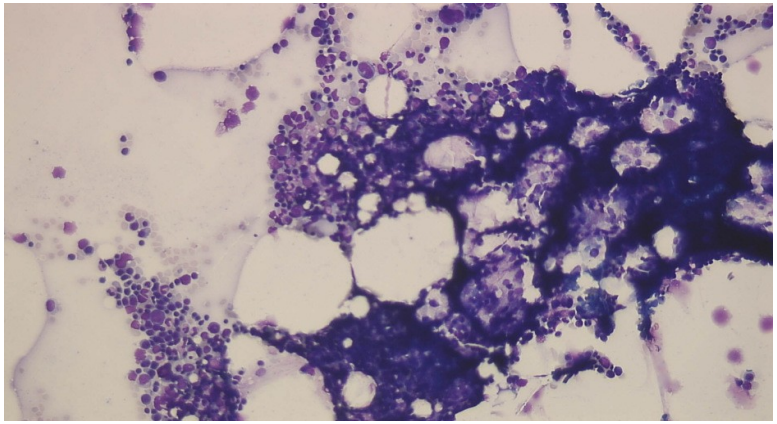
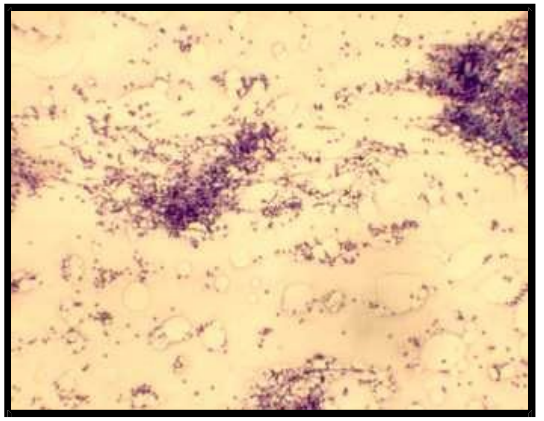




- Cellularity should not be based on aspirate
- fragments and trails are hypocellular
- variable amounts of residual hemopoietic cells
- prominent fat spaces
- megakaryocytes and granulocytic cells are:
  - reduced or absent
  - without dysplasia

Bone Marrow Examination

Required:

- bone marrow aspirate
- trephine biopsy should be done

A trephine is crucial to assess:

- overall cellularity
- topography of hemopoietic cells to exclude an abnormal infiltrate

Bone marrow cellularity is age dependent

Table 1
Characterization of patients

Age (years)	Number of cases	Male/female	Bone marrow cellularity (%)[a]
0–9	9	6/3	60.0 ± 20.0[b]
10–19	13	4/9	56.5 ± 4.4
20–29	12	7/5	54.6 ± 4.6
30–39	11	4/7	54.6 ± 4.6
40–49	10	6/4	54.6 ± 18.2
50–59	9	9/0	52.4 ± 9.5
60–69	12	6/6	58.3 ± 8.3
70–79	13	9/4	56.5 ± 8.7
80–100	11	3/8	41.2 ± 5.9
Total	100	54/46	

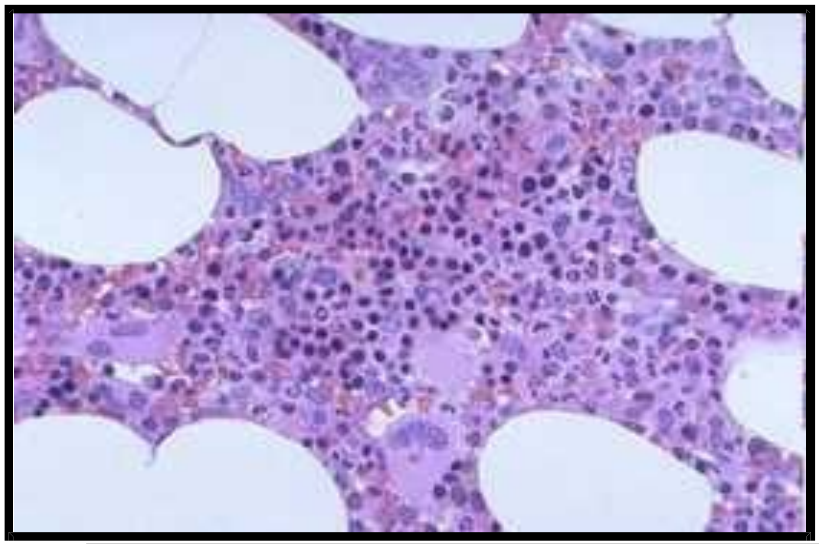
[a] Bone marrow cellularity was measured by the image analyzing system and determined by the percentage of cellular marrow, represented by the formula: (area of hematopoietic cells)/(total area of bone marrow examined) × 100 (%).
[b] Values presented as mean ± S.E.M.

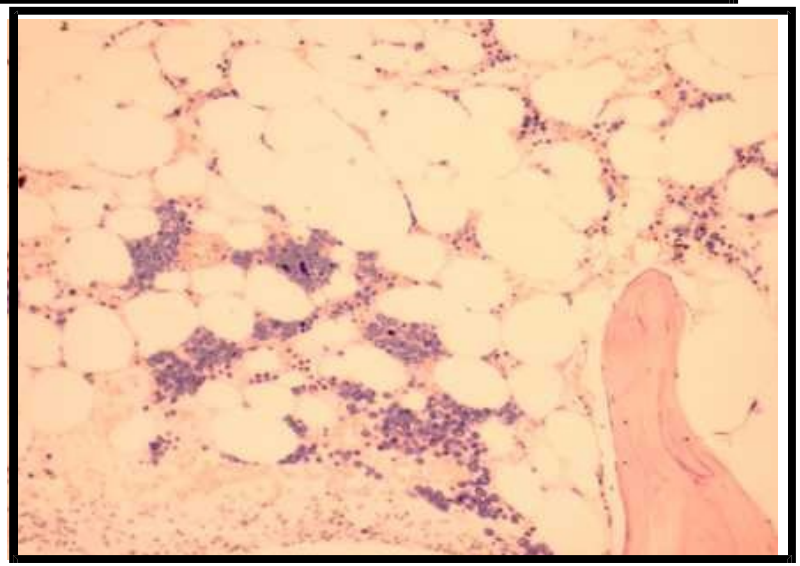
Proliferation stable Apoptosis: ↑ ageing

Ogawa et al. Mechanisms of Ageing and Develop 117 (2000) 57-68

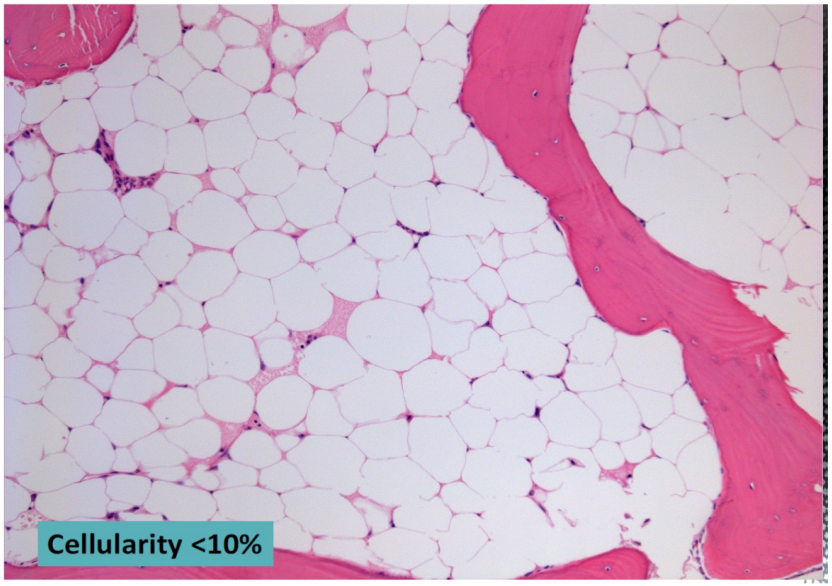
Bone Marrow Histology

Normal cellularity





Hypocellularity (<30%) (rather than aplastic)



Is hypoplastic MDS a distinct entity?

- Patients tend to be younger (< 60y)
- Have profound neutropenia and thrombocytopenia
- Have a lower percentage of blasts
- They less likely display abnormal karyotype
- They usually have a more favourable course
- They usually respond to IST

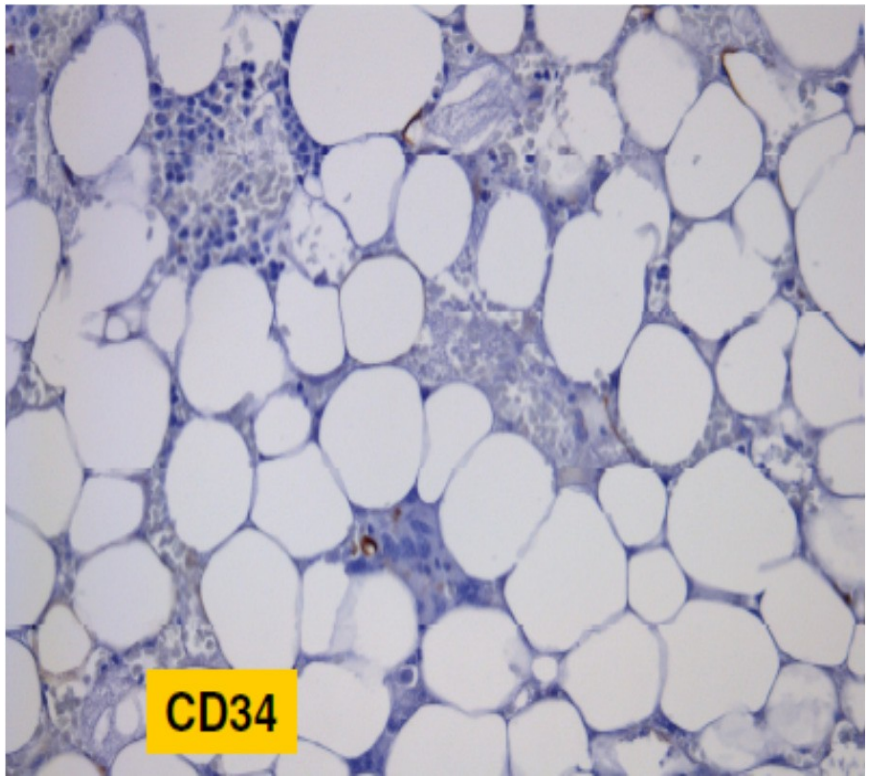
Distinction between AA and hMDS

Characteristics	AA	hypoplastic MDS
dyserythropoiesis	sometimes	yes
abnormal neutrophil	no	yes
dysplastic megakaryocytes	no	yes
fibrosis	no	occasional
increased blasts	no	Sometimes (ALIPS)
CD34+ cells in BM	< 1.0%	sometimes increased
splenomegaly	absent	occasional

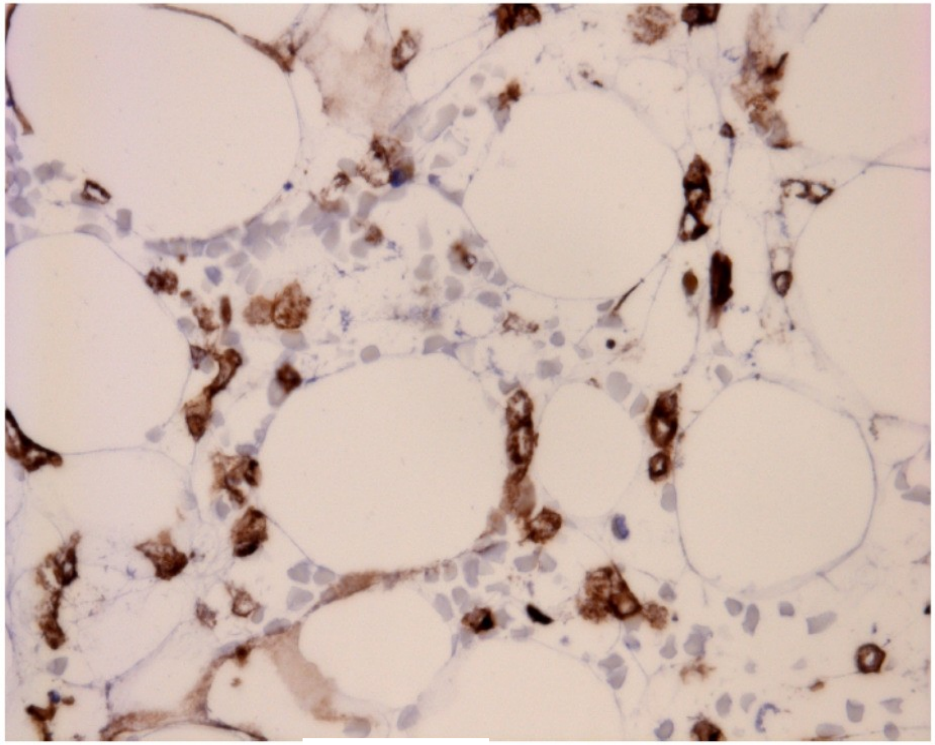
Bennett et al. Sem Hemato 2000;37:15-29 Bennett & Orazi. Haematologica 2009 Feb; 94(2):264-843-70;

CD34+ cells

Aplastic Anemia



Hypoplastic MDS



Cytogenetic investigations and hMDS

- Due to hypocellular bone marrow frequently insufficient metaphases FISH for chr 5 and 7 should be considered
- Isolated del(13q) favorable long-term outcome
- Cytogenetic abnormalities can be present in up to 12%

  of typical AA patients

Socie et al. Seminars in Hematol 2000;37:91-100 Gupta V et al. BJH 2006;34:95-99

Hosokawa et al. Haematologica. 2012;97(12):1845-9.

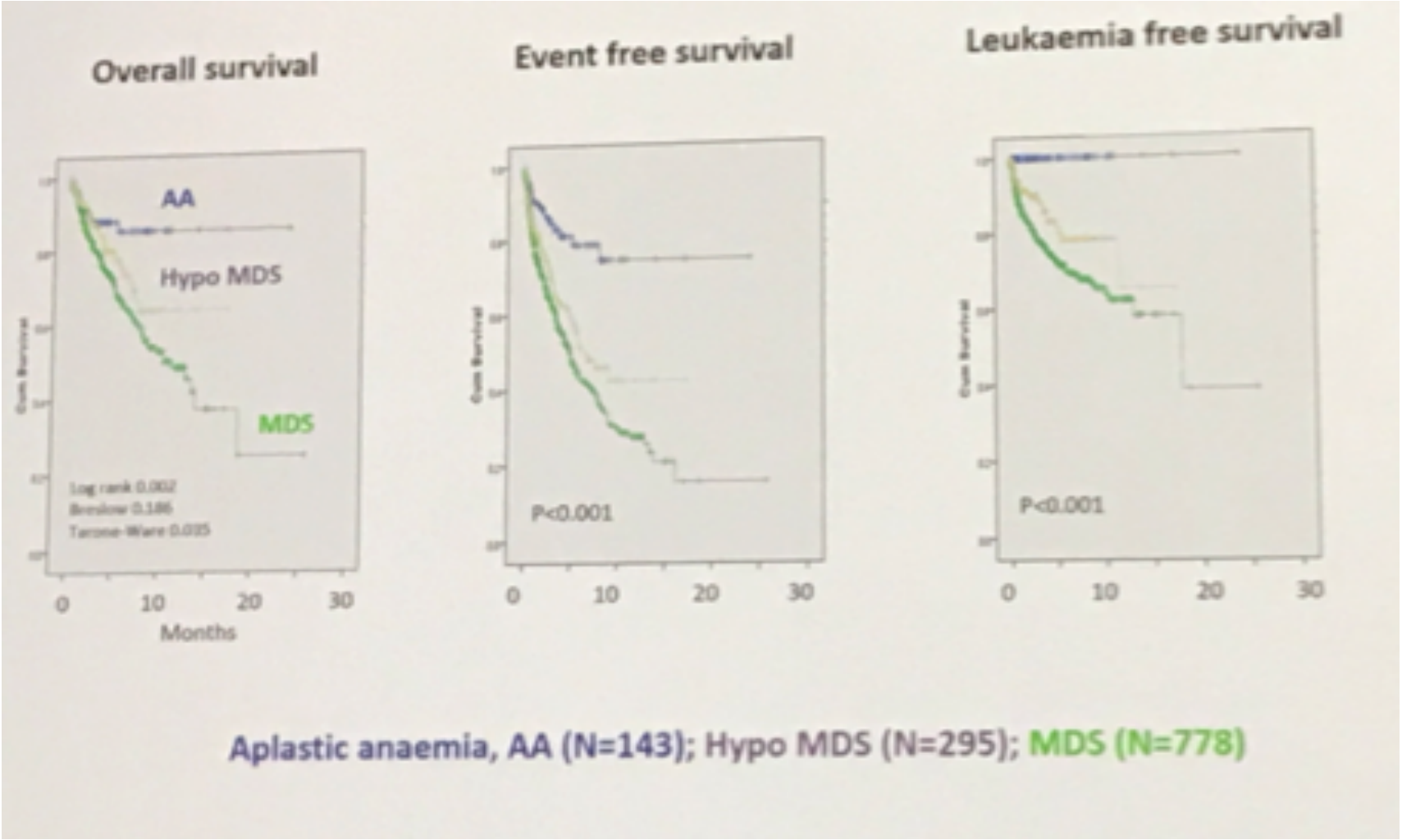
Characteristics of hypocellular MDS

- Hypocellular MDS defined by age
- Frequency: 27% of all MDS patients

	Aplastic anaemia* (N=143)	Hypocellular MDS (N=295)	MDS (N=778)
Age (yr)	42 (16-80)	58 (17-87)	66 (16-92)
ANC ($x10^9$/l)	1.03 (0-6)	1.31 (0-10)	1.75 (0-18)
Platelets ($x10^9$/l)	31 (2-228)	70 (1-650)	104 (2-973)
PNH (granulocytic clone)	43%	19%	4%
BM cytogenetic abnormality	7.3%	36%	39%
Somatic mutation	17%	39%	72%
> 1 mutation	2%	17%	40%

Modified from Mufti G, MDS 2017, oral presentation

Survival according to disease category



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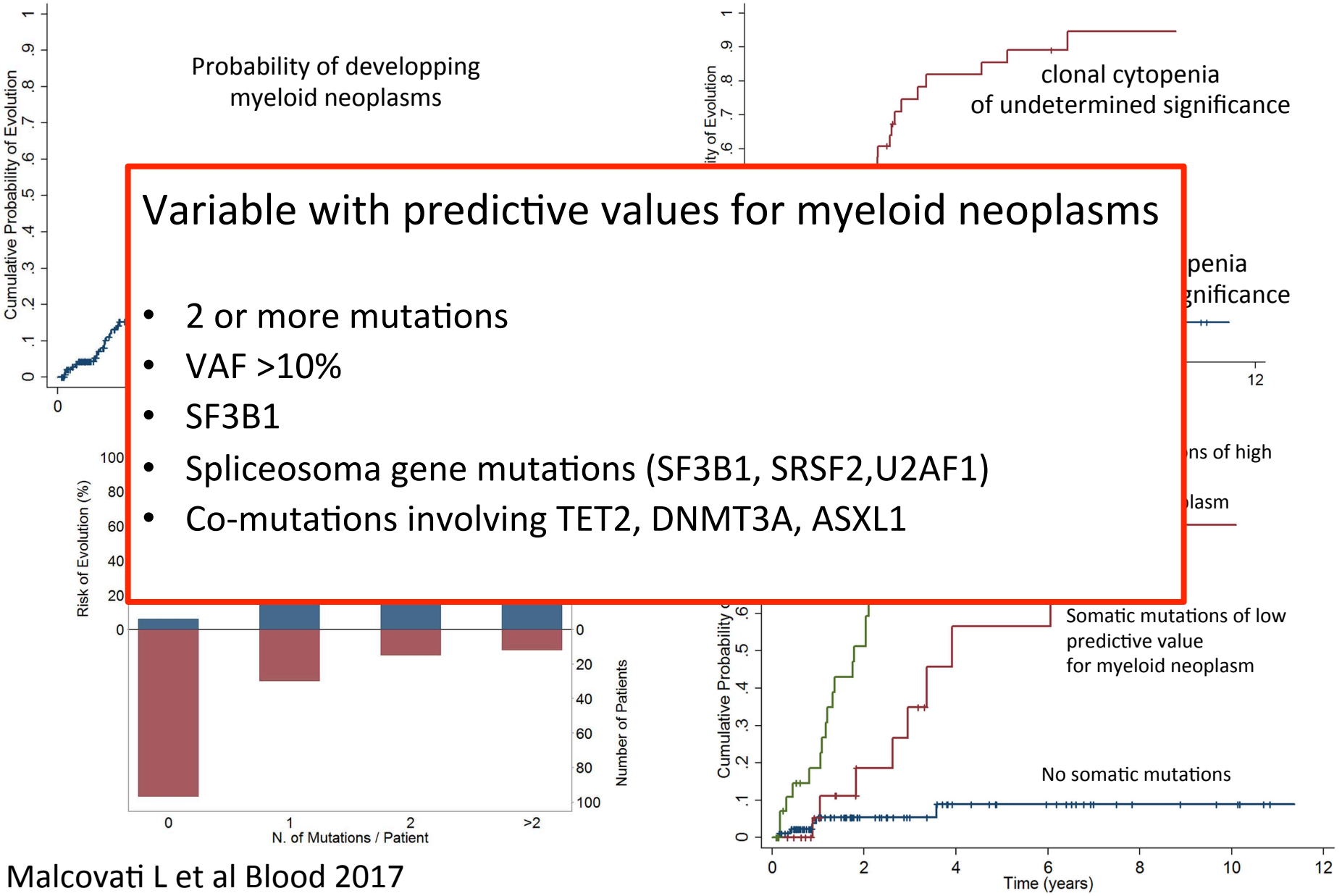
ICUS: Probability of progression to MDS/AML



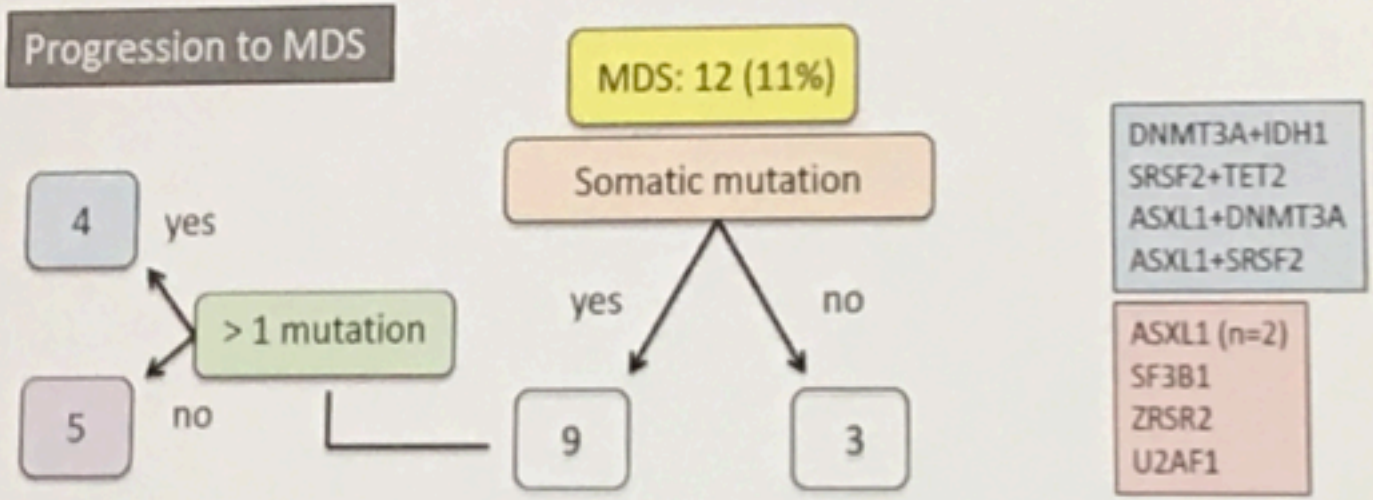
Variable with predictive values for myeloid neoplasms

- 2 or more mutations
- VAF >10%
- SF3B1
- Spliceosoma gene mutations (SF3B1, SRSF2,U2AF1)
- Co-mutations involving TET2, DNMT3A, ASXL1

Malcovati L et al Blood 2017

'Hypocellular ICUS': King's College London/Pavia study

Characteristics (N = 106)	
Age (years)	46 (17-86)
Hb (g/L)	129 (102-163)
ANC $x10^9$/L	1.7 (0.38-9.2)
Platelets $x10^9$/L	153 (20-335)
BM chromosomal abnormality	5/96 (5.2%)
Somatic mutation	19/95 (20%)
>1 mutation	6/95 (6.3%)



Bono E et al, manuscript in preparation

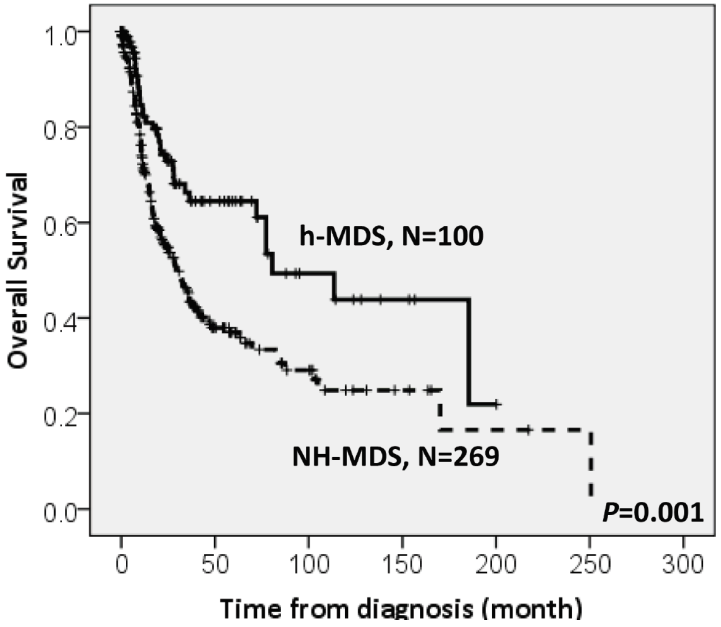
Modified from Mufti G, MDS 2017, oral presentation

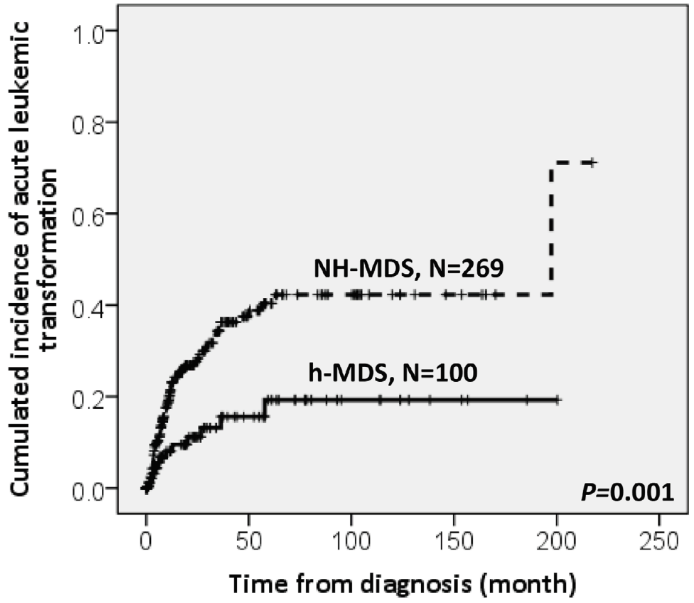
Research Paper

Distinct mutation profile and prognostic relevance in patients with hypoplastic myelodysplastic syndromes (h-MDS)

Chi-Yuan Yao[1,*], Hsin-An Hou[1,*], Tzung-Yi Lin[1], Chien-Chin Lin[1,2], Wen-Chien Chou[1,2], Mei-Hsuan Tseng[1], Ying-Chieh Chiang[1], Ming-Chih Liu[3], Chia-Wen Liu[3], Yuan-Yeh Kuo[4], Shang-Ju Wu[1], Xiu-Wen Liao[5], Chien-Ting Lin[1,5], Bor-Shen Ko[1], Chien-Yuan Chen[1], Szu-Chun Hsu[2], Chi-Cheng Li[5], Shang-Yi Huang[1], Ming Yao[1], Jih-Luh Tang[1,5], Woei Tsay[1], Chieh-Yu Liu[6], Hwei-Fang Tien[1]

……Our findings provide evidence that h-MDS indeed represent a distinct clinico-biological subgroup of MDS and can predict better leukemia-free survival and OS.





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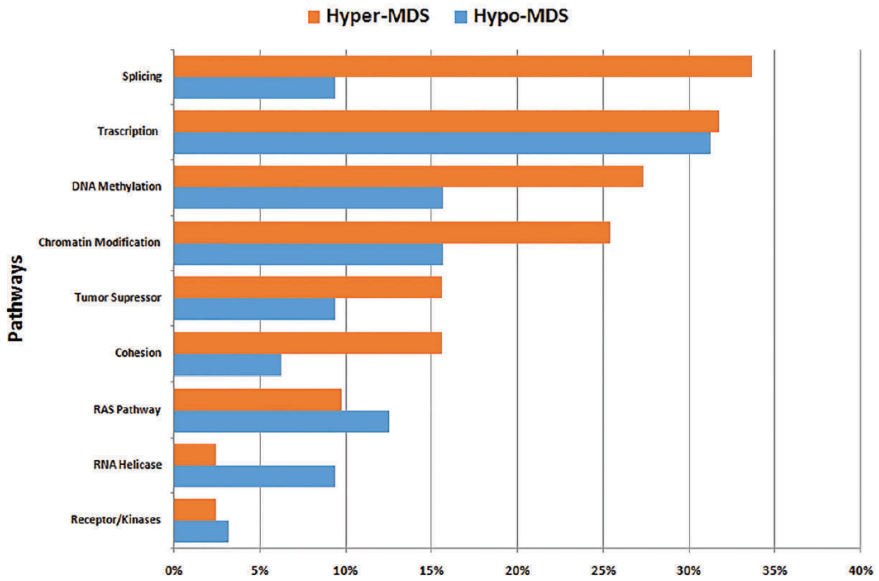
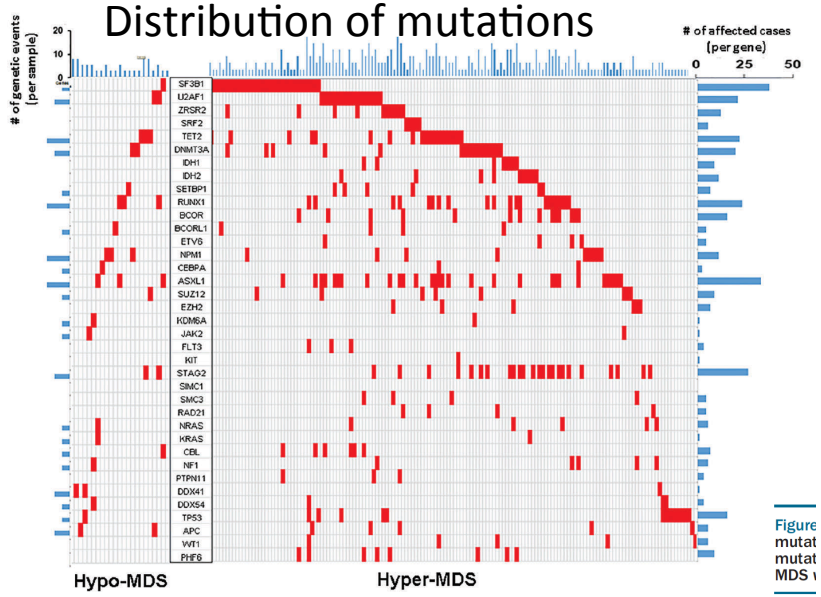
Variable	RR	Overall survival		*P* value
		Lower 95% CI	Upper 95% CI	
Age[†]	1.533	1.035	2.273	0.033*
Sex	1.353	0.957	1.912	0.087
BM Hypocellularity	0.655	0.431	0.995	0.047*
IPSS-R[ζ]	3.431	2.404	4.896	<0.001*
TP53	5.904	3.442	10.130	<0.001*
ASXL1	1.394	0.892	2.179	0.144
EZH2	1.066	0.479	2.371	0.876
DNMT3A	1.367	0.847	2.204	0.200
RUNX1	0.983	0.608	1.590	0.946
SRSF2	1.440	0.838	2.474	0.187
HSCT or intensive				

Somatic mutations and hMDS

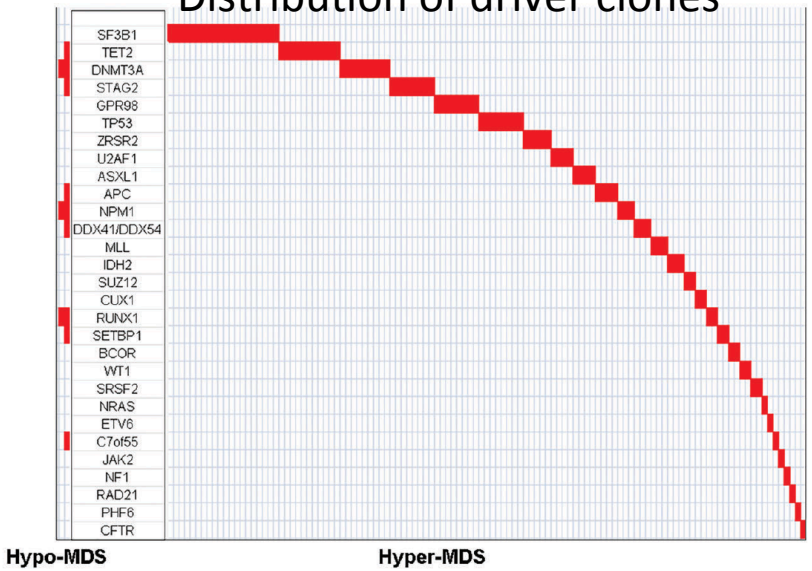
Frequency of gene mutations involved in common functional pathways



Distribution of mutations

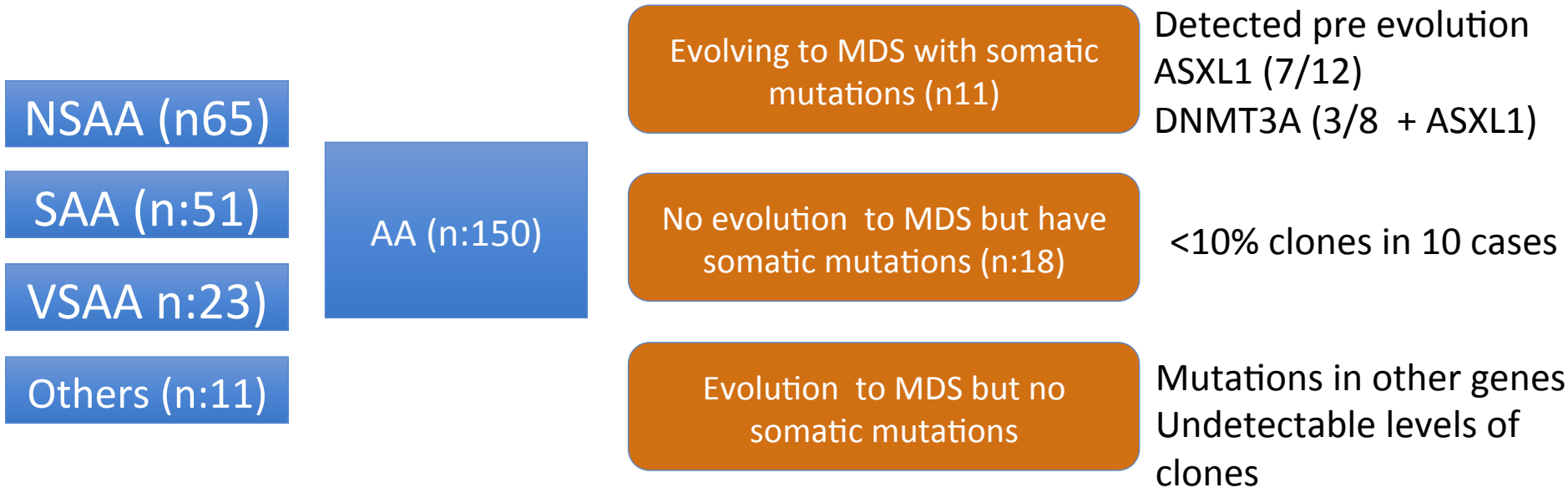


Distribution of driver clones



Nazha et al Haematologica 2015, 100:e437

Somatic mutations identify a subgroup of aplastic anemia patients who progress to myelodysplastic syndrome



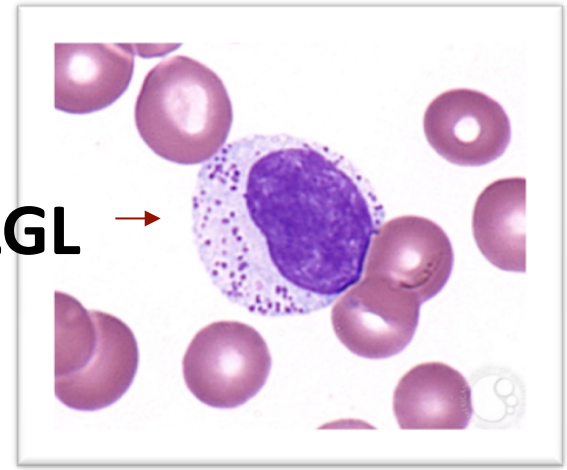
Somatic mutations found in 29/150 (19%)
In presence of somatic mutations the risk of MDS is 38% vs 6%

KULASEKARARAJ et al BLOOD, 23 October 2014, Volume 124, Number 17

e l’LGL proliferations?

LA LEUCEMIA A GRANDI LINFOCITI GRANULATI

Raro disordine linfoproliferativo cronico caratterizzato dall'espansione dei grandi linfociti granulati nel sangue periferico



Grandi Linfociti Granulati

- Linfociti con funzione citotossica
- Individuo sano: 10-15% delle cellule mononucleate del sangue periferico (PBMC), range fisiologico tra 0,2 e 0,4x10^9 LGL/L

  Paziente ➔ 25%-95% dei PBMC

Linfociti T citotossici (CTL)

Cellule Natural Killer (NK)

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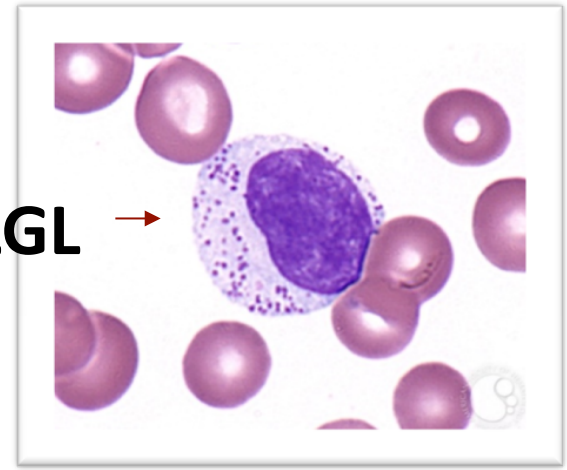

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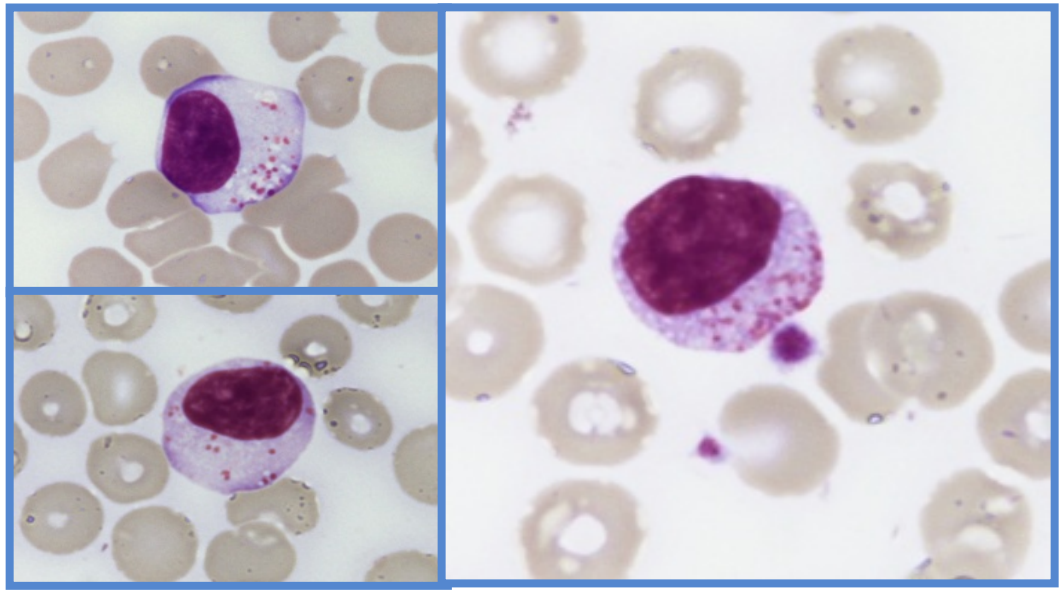
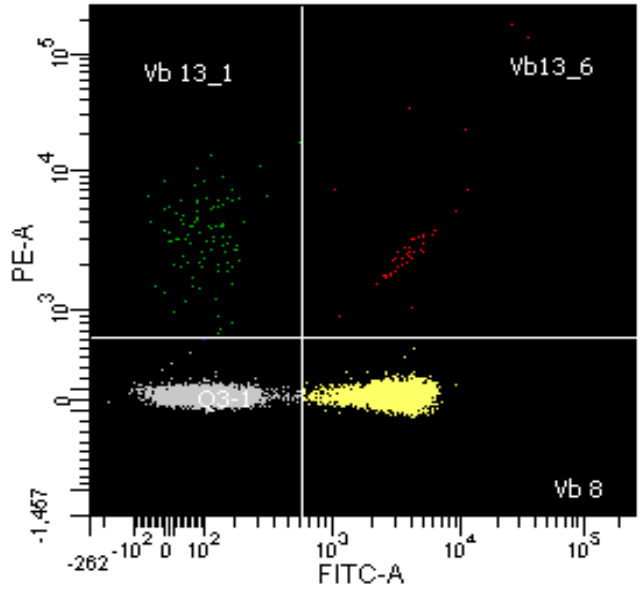
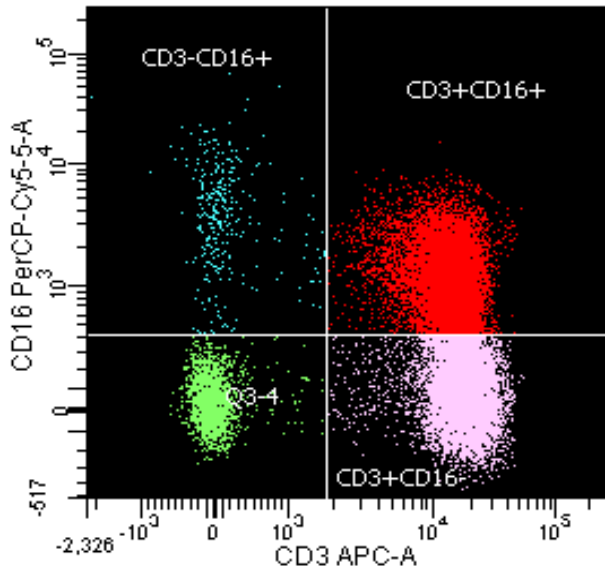
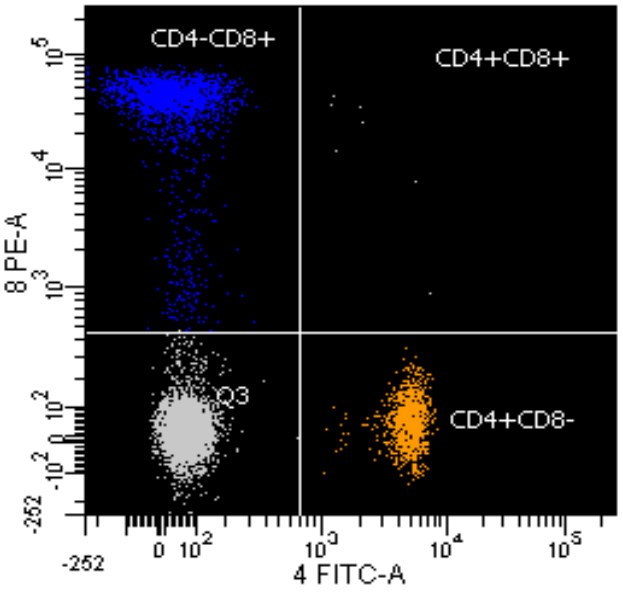
T-LGLL	CLPD-NK
T-cell Large Granular Lymphocyte Leukemia	**Chronic Lymphoproliferative Disorder of NK-cells**
Incidenza: 85%	**15%**

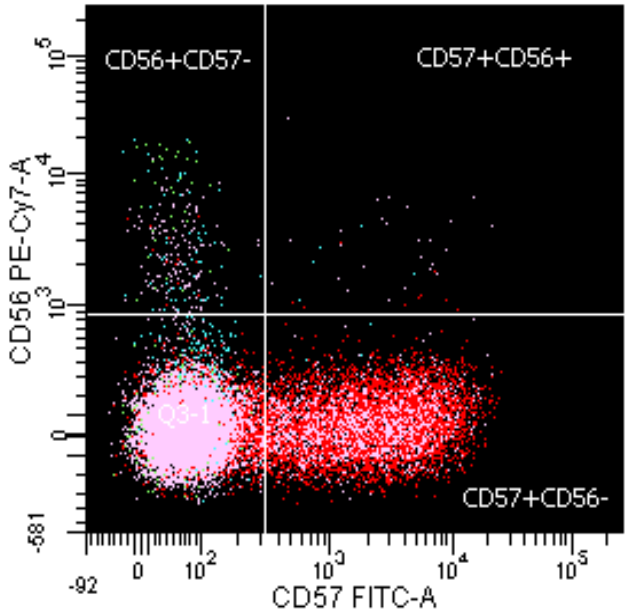
T LGL leukemia



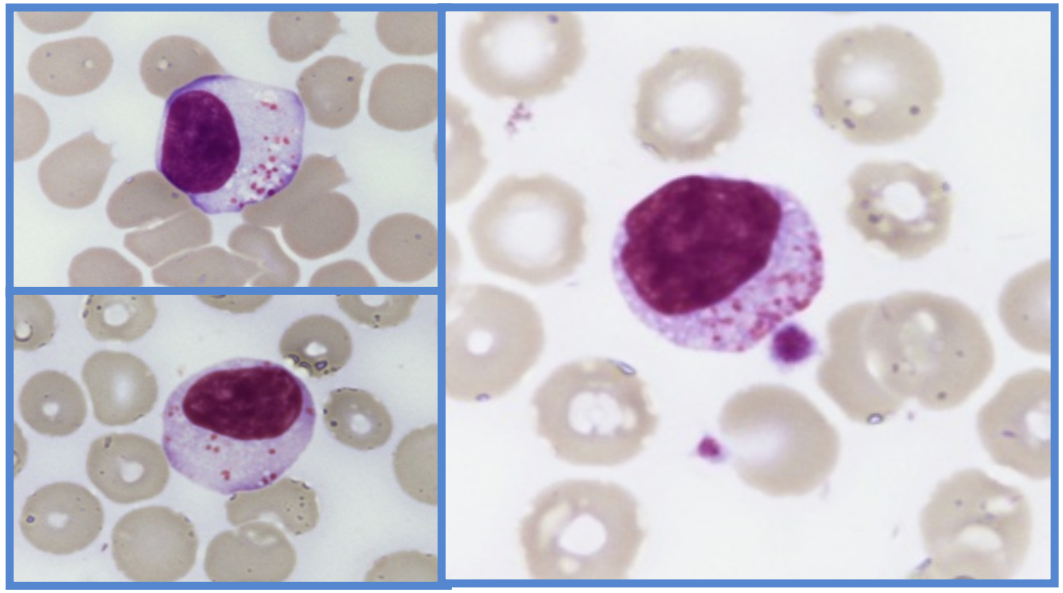


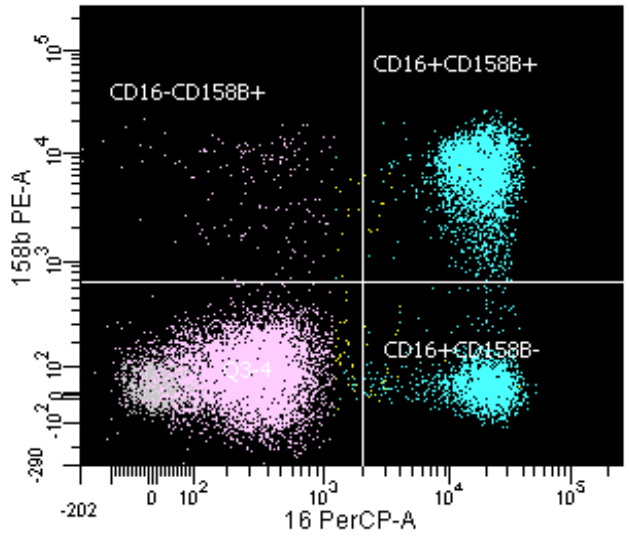


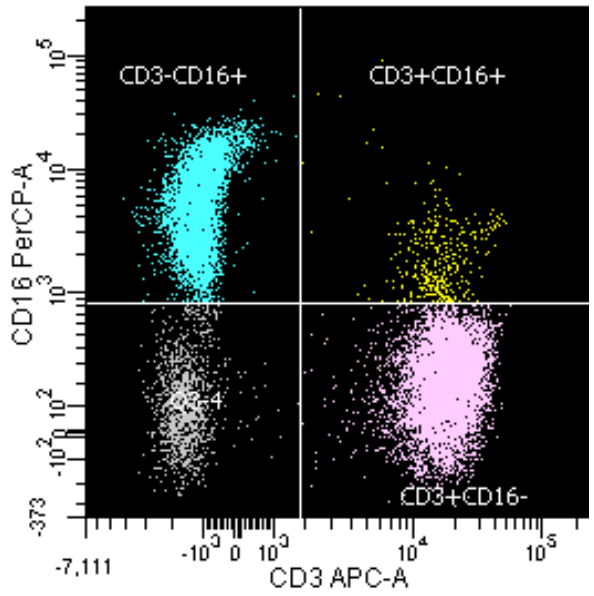


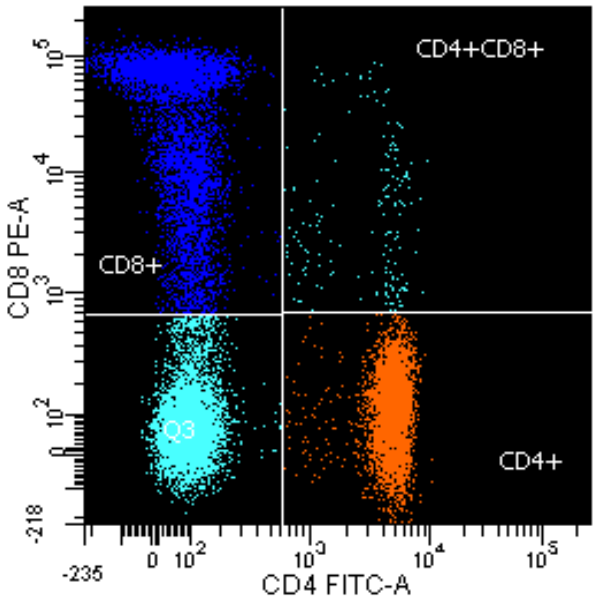


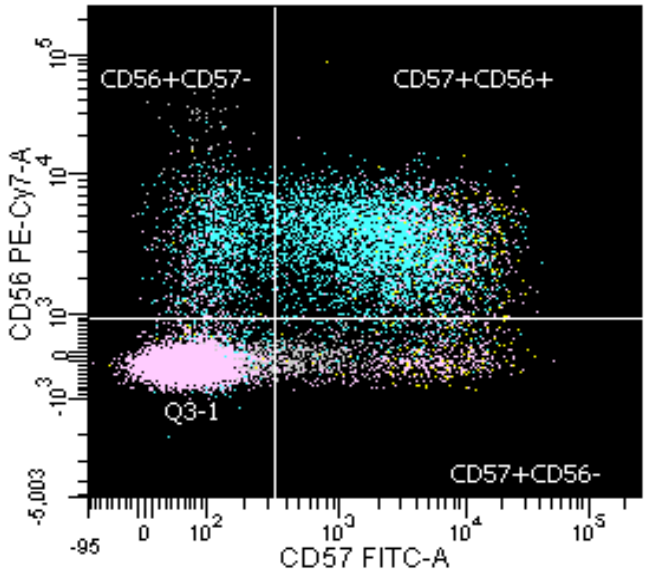
Chronic Lymphoproliferative Disorder of NK cells











LA LEUCEMIA LGL - CARATTERISTICHE

DIAGNOSI

- LGL > 500/uL per un periodo di tempo superiore a 6 mesi
- Presenza di clonalità dell'espansione

CLINICA

- 40% inizialmente asintomatica
- Citopenie (**neutropenia 80%**)
- Splenomegalia
- Astenia e sintomi B
- Associazione con altre malattie

Associated diseases with LGL leukemia	Frequency
Neoplasms	4 to 10%
Autoimmune cytopenia PRCA AIHA ITP Evans syndrome	5 %
B-cell lymphoid neoplasms Low-grade NHL DLBCL Mantle cell lymphoma Multiple myeloma CLL Hairy cell leukemia Waldenstrom macroglobulinemia Hodgkin lymphoma Lymphomatoid granulomatosis Heavy chain disease	5%
Autoimmune diseases/connective tissue disorders Rheumatoid arthritis Systemic lupus erythematosus Vasculitis Systemic sclerosis Endocrinopathy APECED Type I MEN Hashimoto Grave disease CIBD Celiac disease Gougerot-Sjogren syndrome Glomurolenephritis Polymyositis Inclusion body myositis Poly/multinevritis RPA Inflammatory arthritis (unclassified) Lambert-Eaton myasthenic syndrome Good syndrome Behcet disease Multiple sclerosis Acquired factor VIII inhibitor	10 to 20% 10 to 18%
Myelodysplasia	3 to 10%
AML	< 1%
Hemophagocytic syndrome	< 1%
Pulmonary hypertension	< 1%
Post organ or hematopoietic stem cell transplant	< 1%
Post viral infection	< 1%

Brief report

Lymphoproliferative disease of granular T lymphocytes presenting as aplastic anemia

Ronald S. Go, Ayalew Tefferi, Chin-Yang Li, John A. Lust, and Robert L. Phyliky

(Blood. 2000;96:3644-3646)

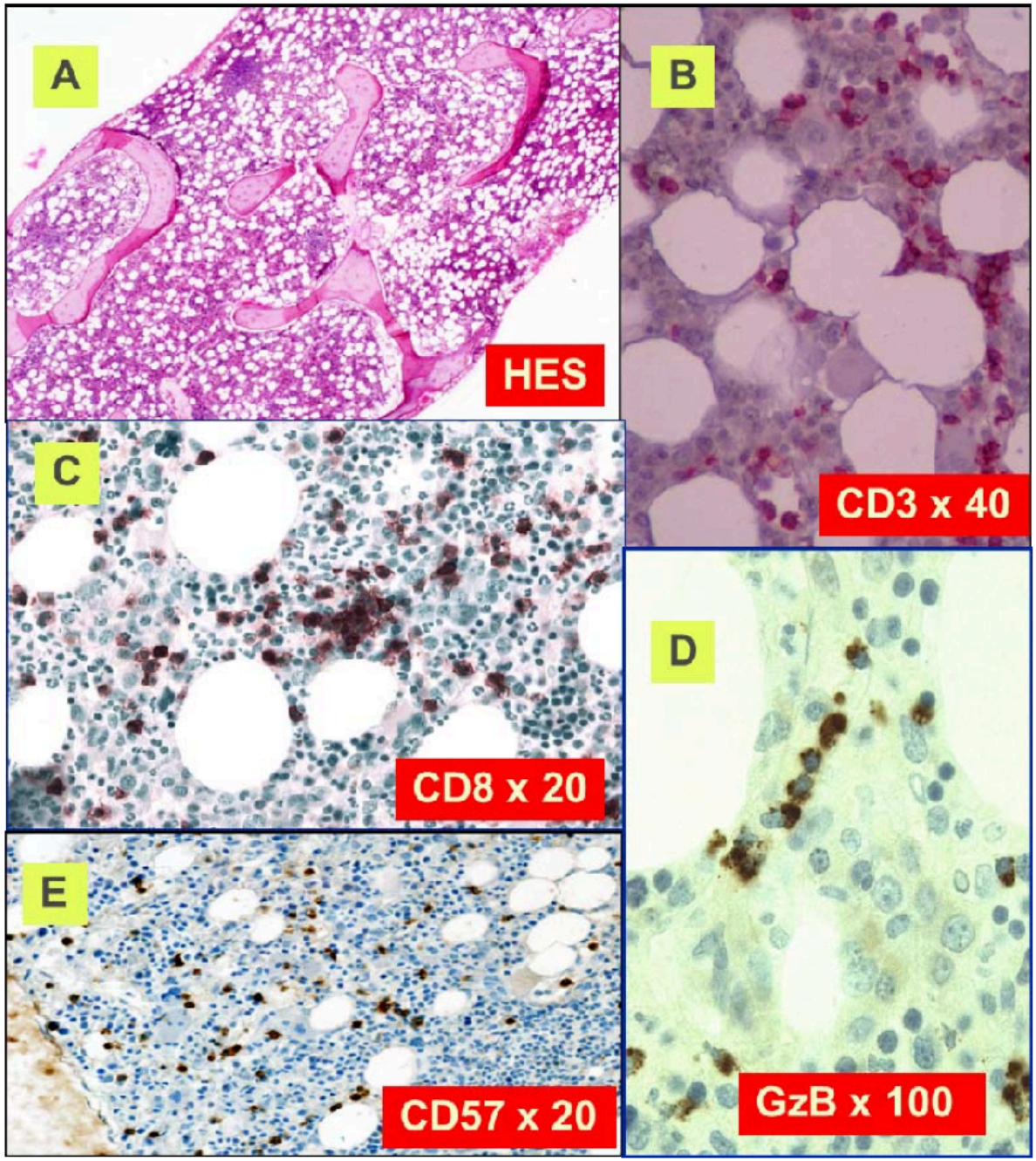
T-Cell Large Granular Lymphocyte Leukemia Associated With Myelodysplastic Syndrome

A Clinicopathologic Study of Nine Cases

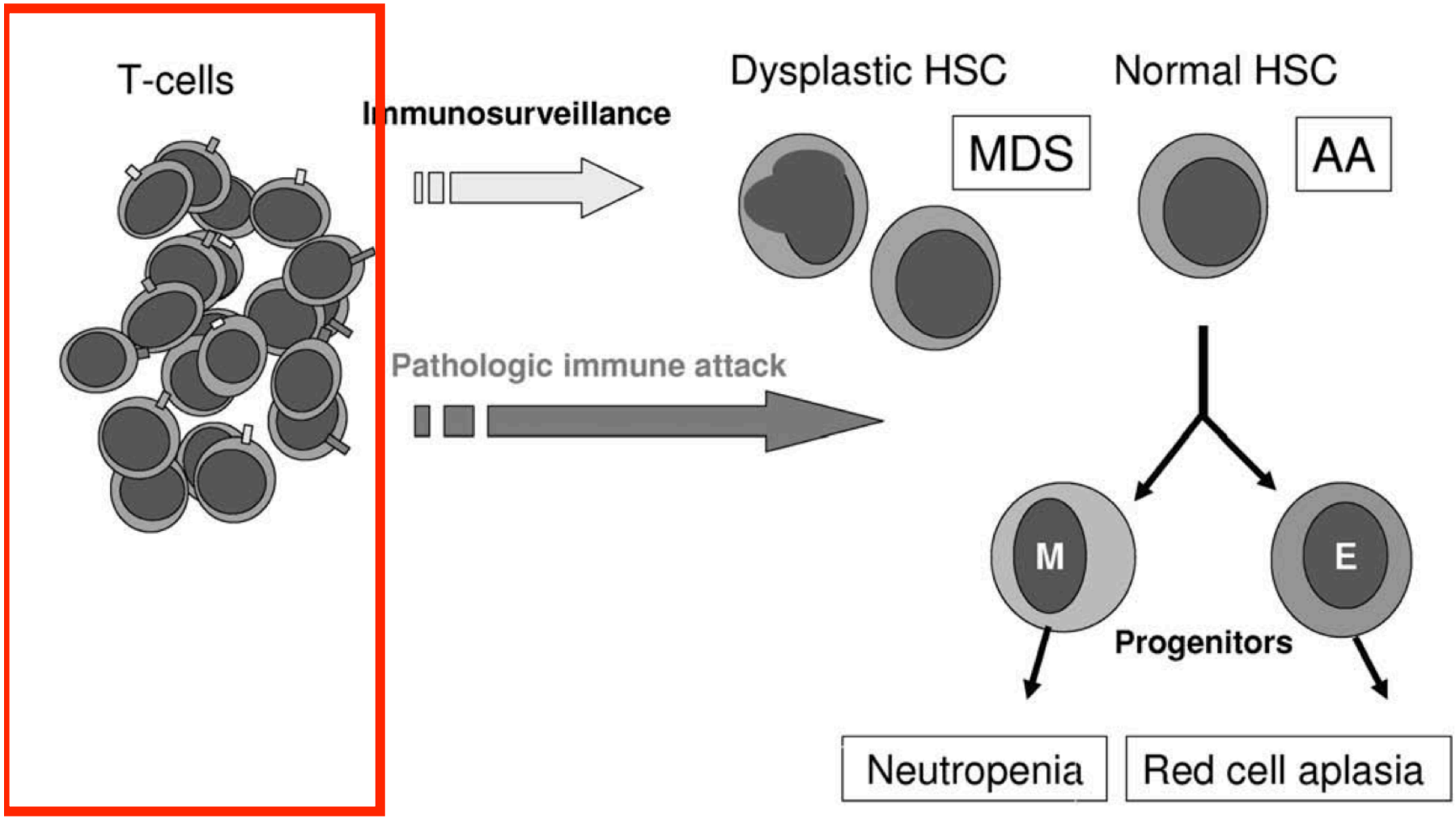
Yang O. Huh, MD,[1] L. Jeffrey Medeiros, MD,[1] Farhad Ravandi, MD,[2] Sergej Konoplev, MD,[1] Jeffrey L. Jorgensen, MD, PhD,[1] and Roberto N. Miranda, MD[1]

Am J Clin Pathol 2009;131:347-356

Bone marrow in LGL



Immune mediated bone marrow failure: effector cells and targets



Maciejewski J P et al FOLIA HISTOCHEMICA ET CYTOBIOLOGICA Vol. 45, No. 1, 2007 p. 5-14

MDS and T cell immune disregulation

- High levels of TNFα and IFNγ have been reported [1,2]
- Expansions of T cell clones with limited TCR-Vβ repertoire [3]
- WT1 protein (overexpressed in MDS with trisomy 8 abnormality)[4]
- Presence of PNH clones[5]

[1]Kitagawa M et al, Leukemia, 1997; 11:2049 [2]Selleri C et al Cancer 2002, 95:1911
[3]Epling-Burnette PK et al, Leukemia 2007; 21:659 [4]Sloand EM et al Blood 2005; 106:841
[5]Maciejewski JP et al Br J Haematol 2001; 115:1015

Limited heterogeneity of T cell receptor BV usage in aplastic anemia

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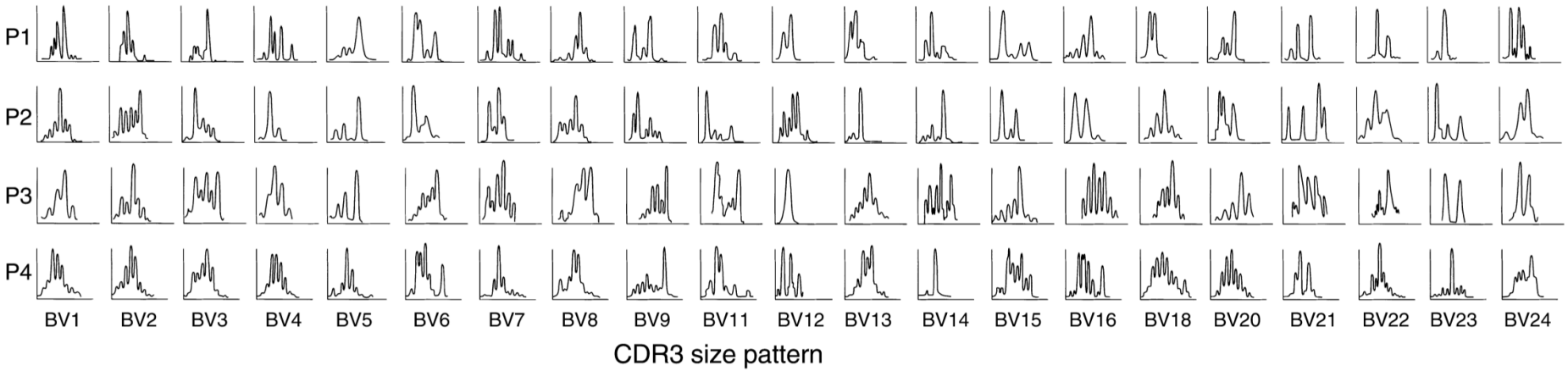
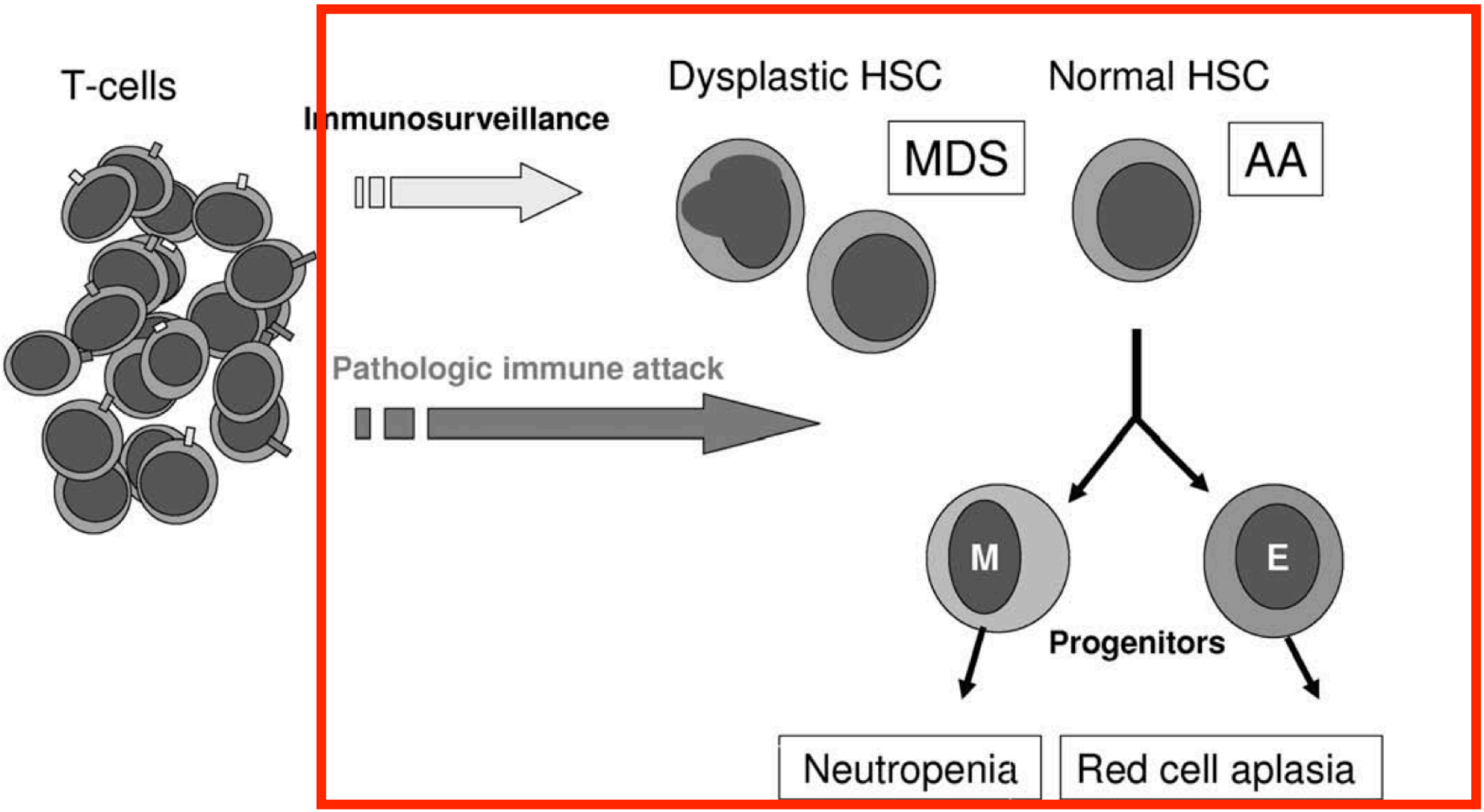
Received for publication March 8, 2001, and accepted in revised form July 14, 2001.



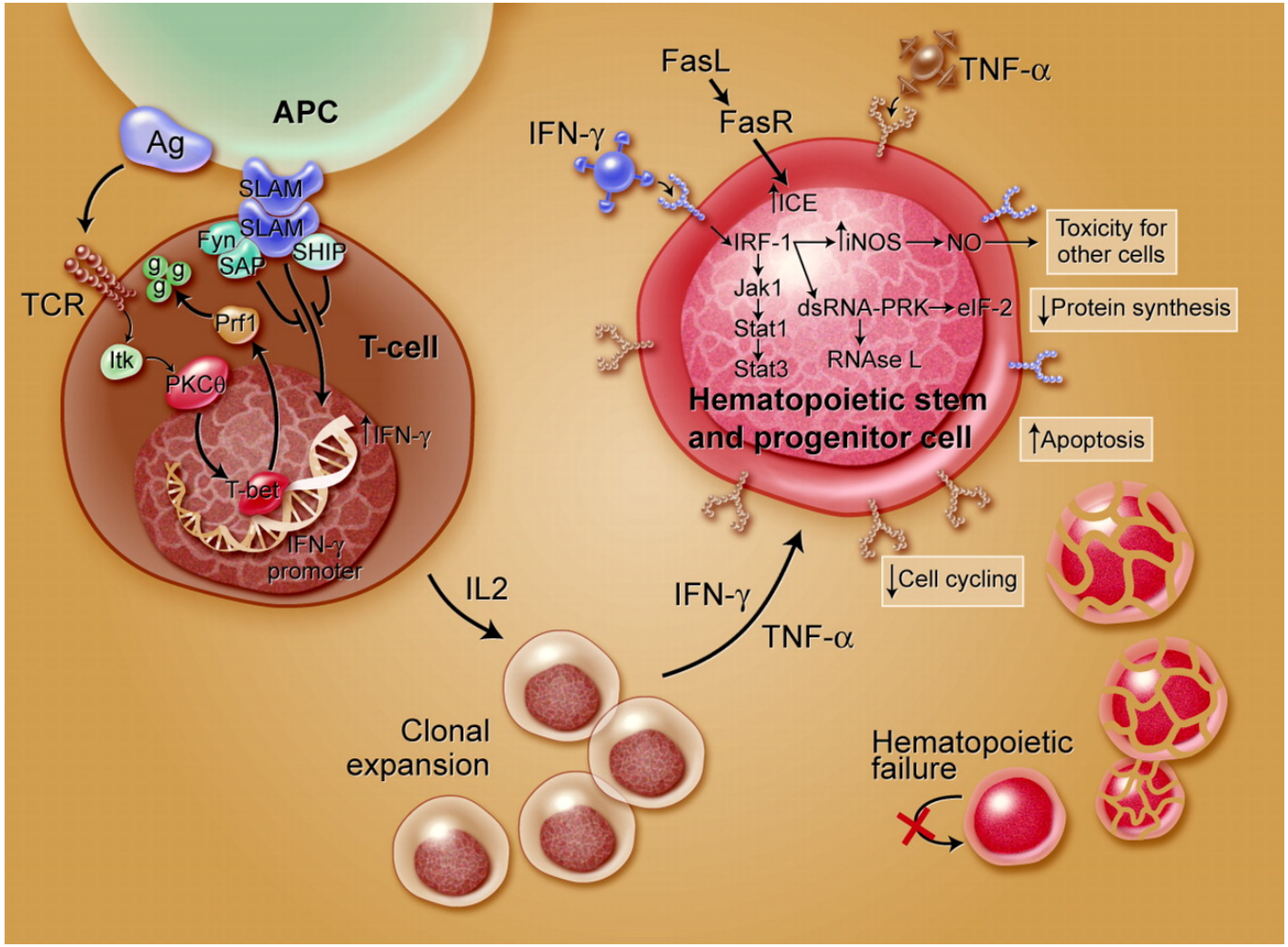
J. Clin. Invest. **108**:765–773 (2001). DOI:10.1172/JCI200112687.

Immune mediated bone marrow failure: effector cells and targets



Maciejewski J P et al FOLIA HISTOCHEMICA ET CYTOBIOLOGICA Vol. 45, No. 1, 2007 p. 5-14

Immune destruction of hematopoiesis in AA

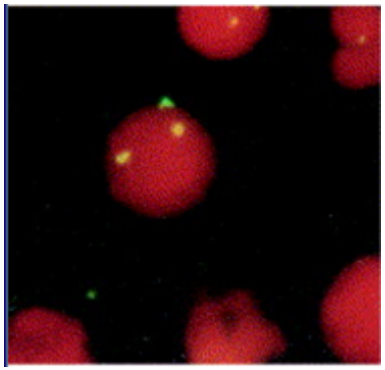


Young et al Blood, 2006; 108: 2509

blood

Molecular model of T cell patogenesis in MDS

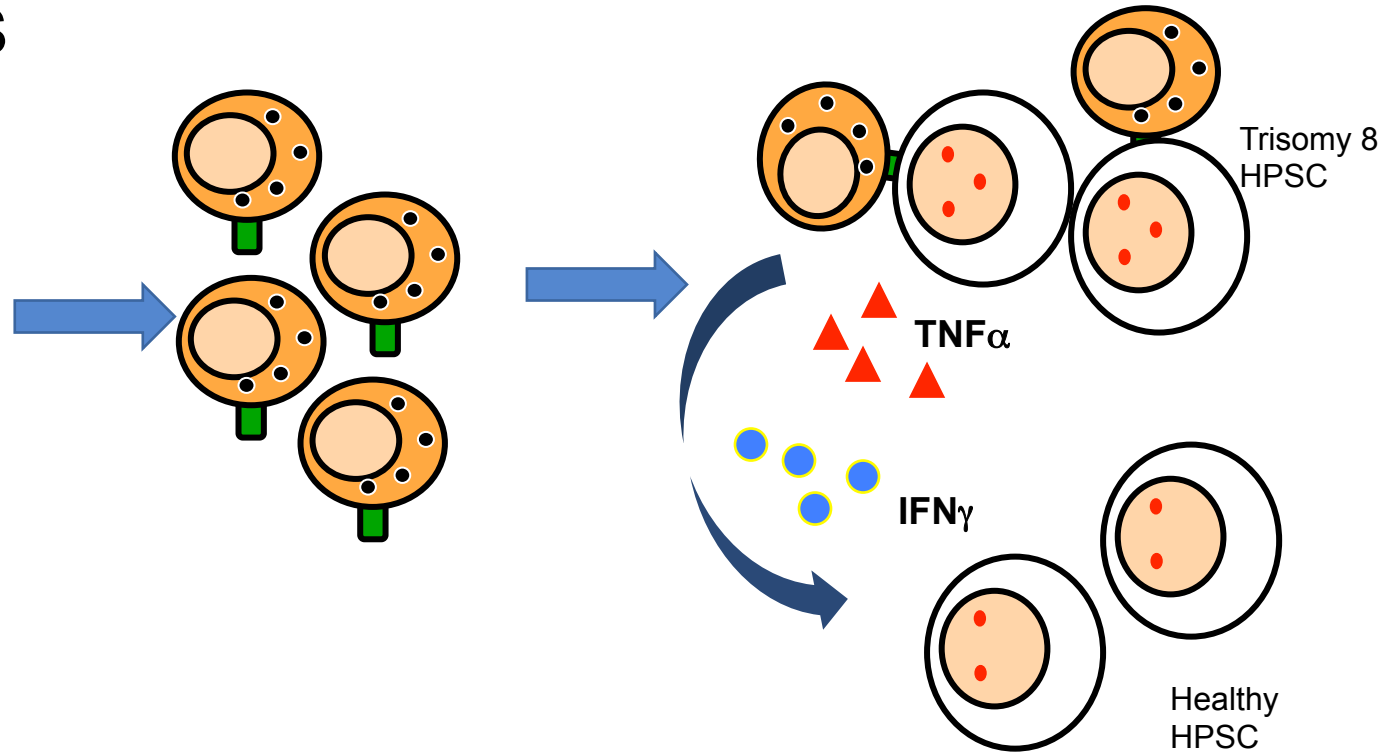
Trisomy 8 MDS



BM HPCs

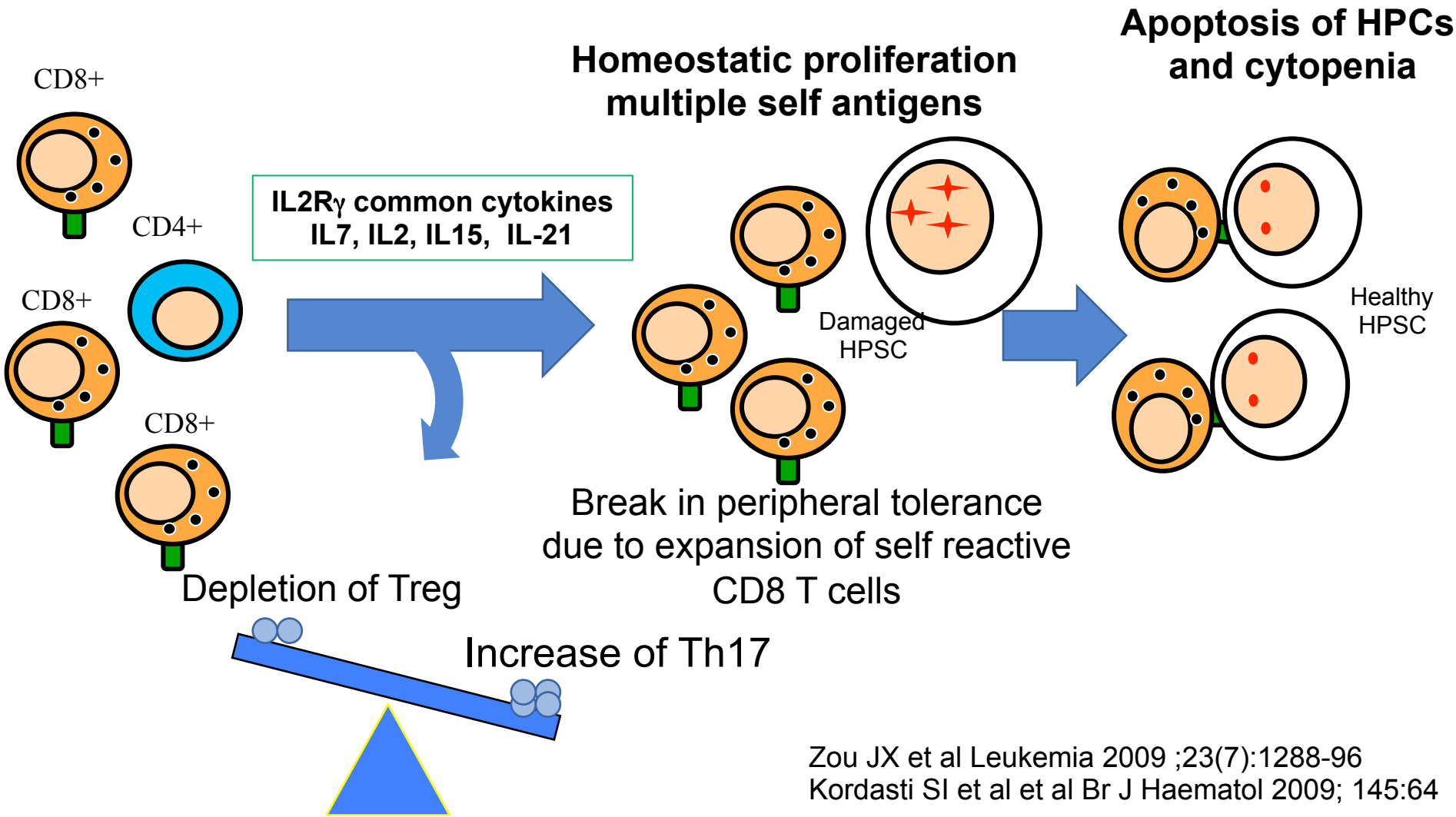
Direct CD8 + antigen specific proliferative response to trisomy 8 antigen (WT1)

T cell mediated suppression of healthy and abnormal bone marrow progenitors



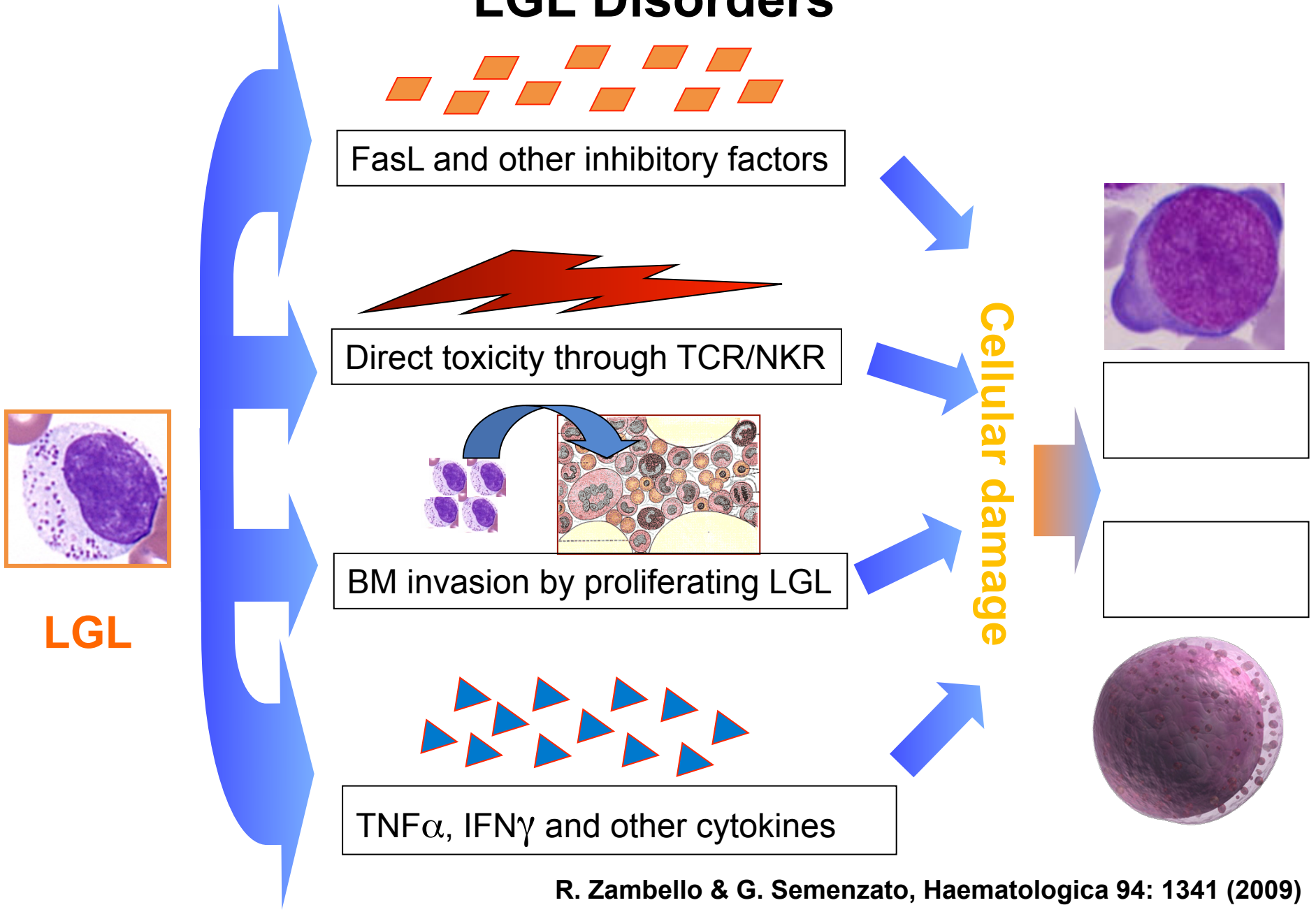
Sloand EM et al Blood 2005; 106:841

Molecular model of T cell pathogenesis in MDS



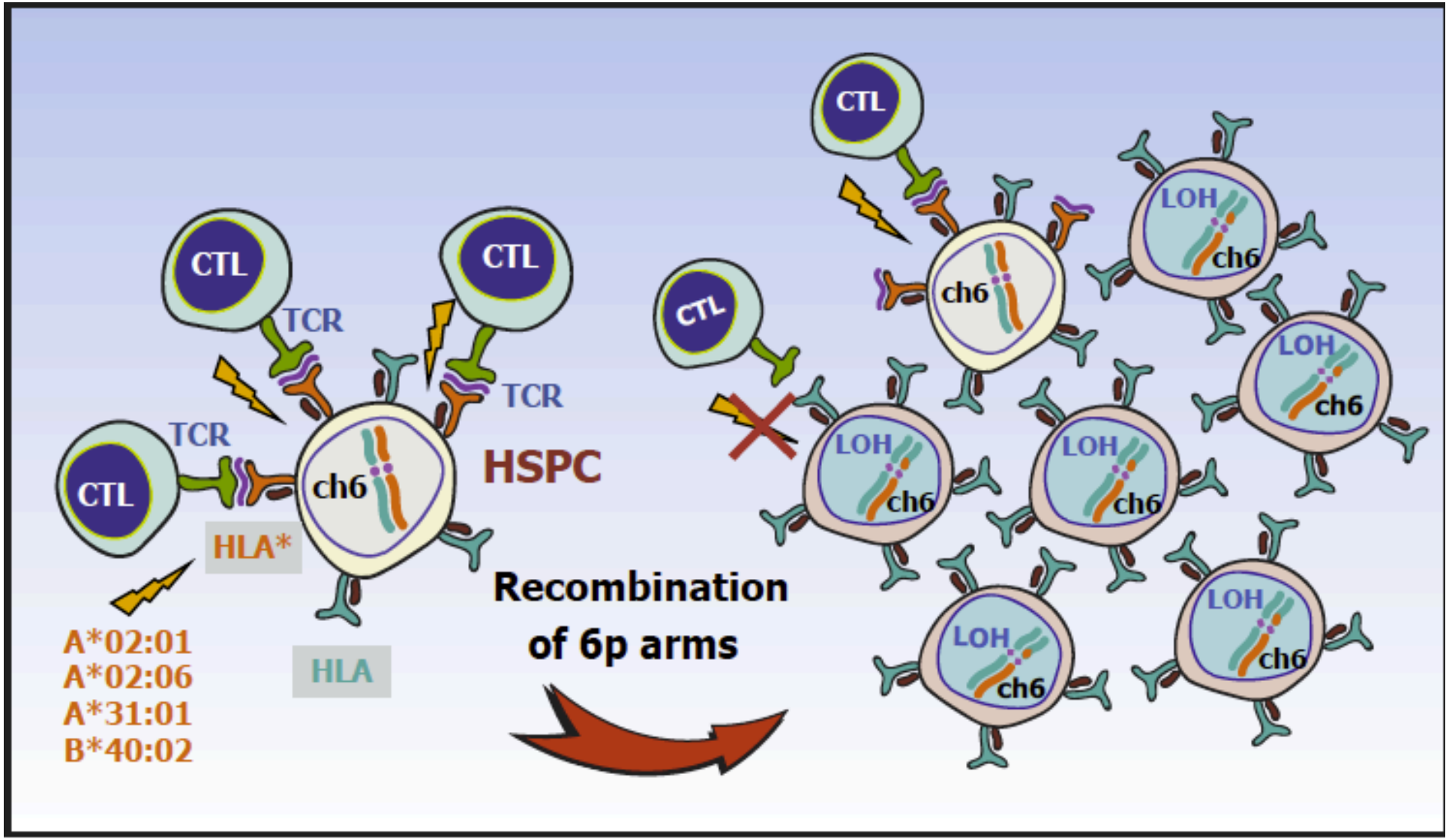
Zou JX et al Leukemia 2009 ;23(7):1288-96
Kordasti SI et al et al Br J Haematol 2009; 145:64

Pathogenetic Hypothesis of Bone Marrow Failure in LGL Disorders



R. Zambello & G. Semenzato, Haematologica 94: 1341 (2009)

Copy number neutral 6p LOH



Katagiri et al Blood. 2011;118(25):6601-6609

Detection and significance of clonal populations with a paroxysmal nocturnal hemoglobinuria (PNH) phenotype

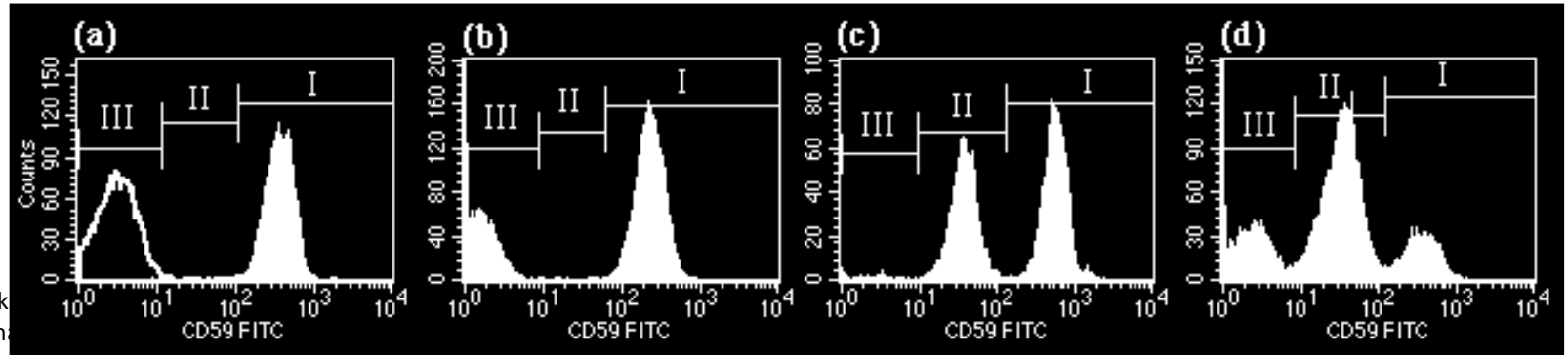
PNH clones are reliably detected in many patients with aplastic anemia, MDS and LGL, although the clone size is generally small (<5%)

PNH

- PIG-A gene on X chromosome
- Single somatic mutation sufficient
- Deficiency of GPI membrane-anchored proteins
- Manifestations:
  - Complement-mediated hemolysis
  - Thrombosis
  - Aplastic anemia
  - Rarely leukemia

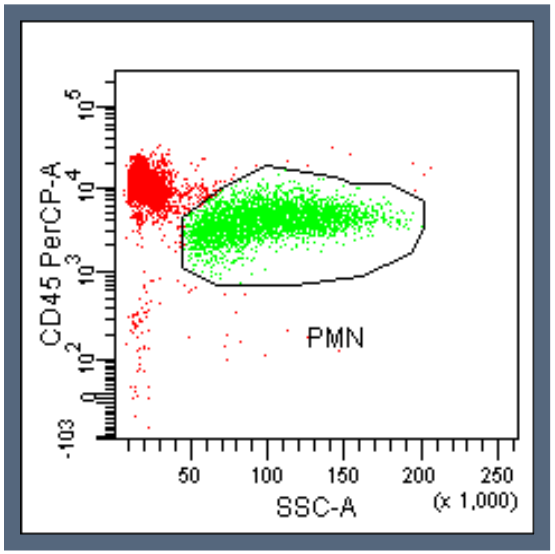
Diagnostic Test for PNH

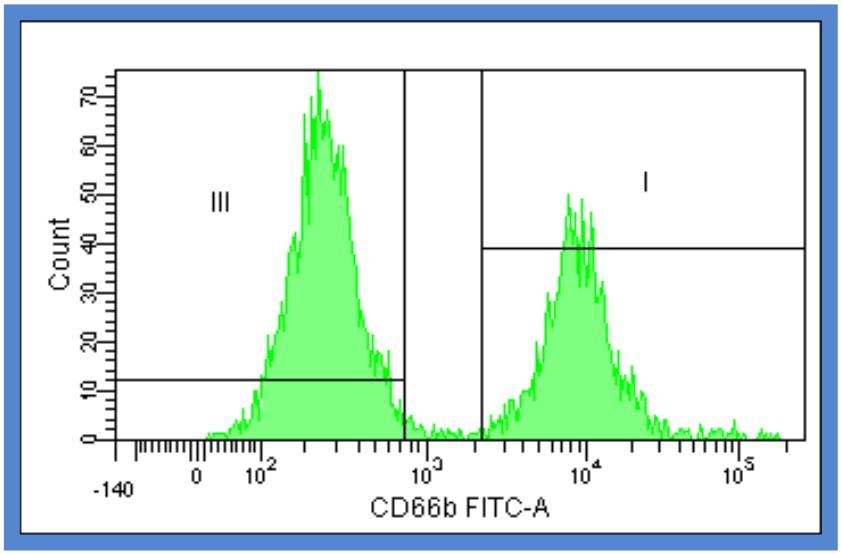
- Flow Cytometry performed on peripheral blood
- Use monoclonal antibodies against GPI-anchored proteins, such as CD59 or CD66b, CD48, CD14 or FLAER

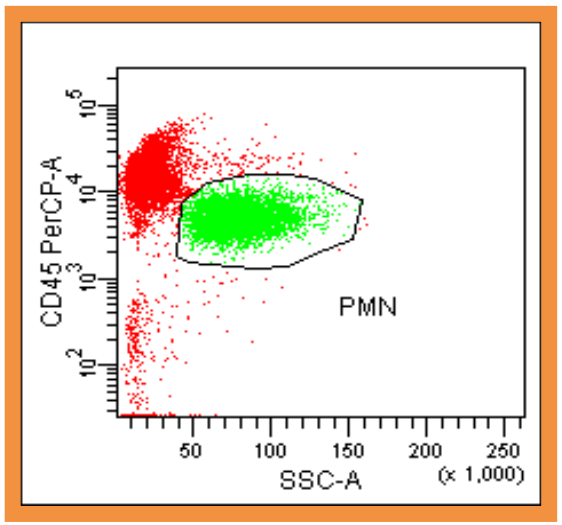


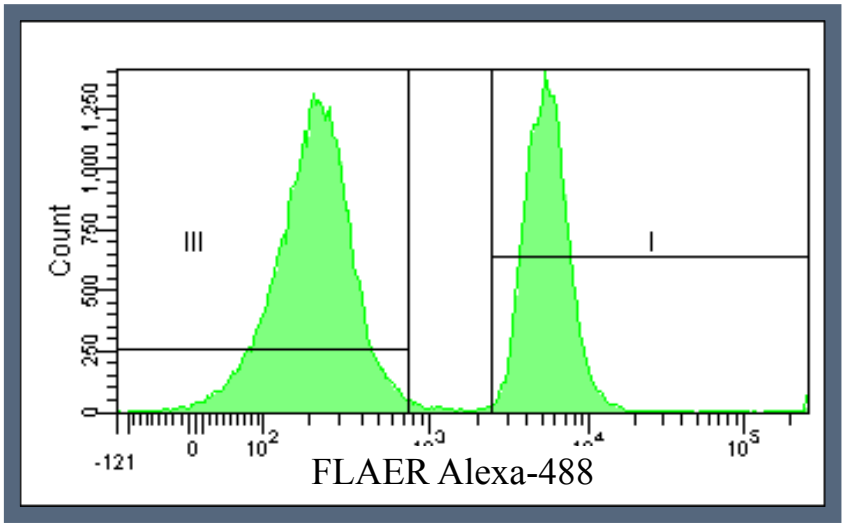
1. Park
2. Rich

Comparison between FLAER and CD66b

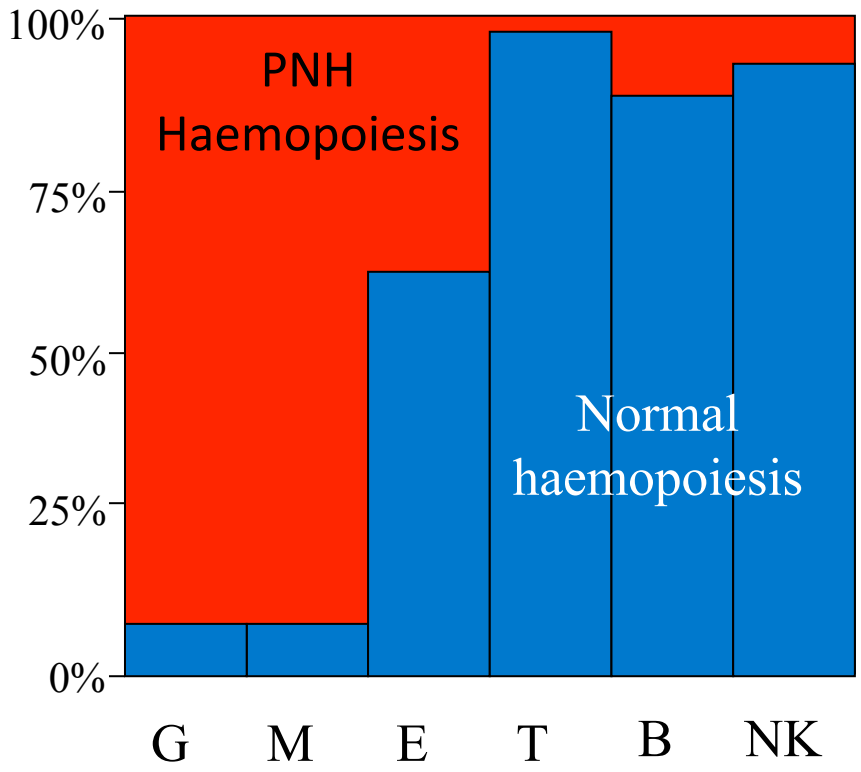




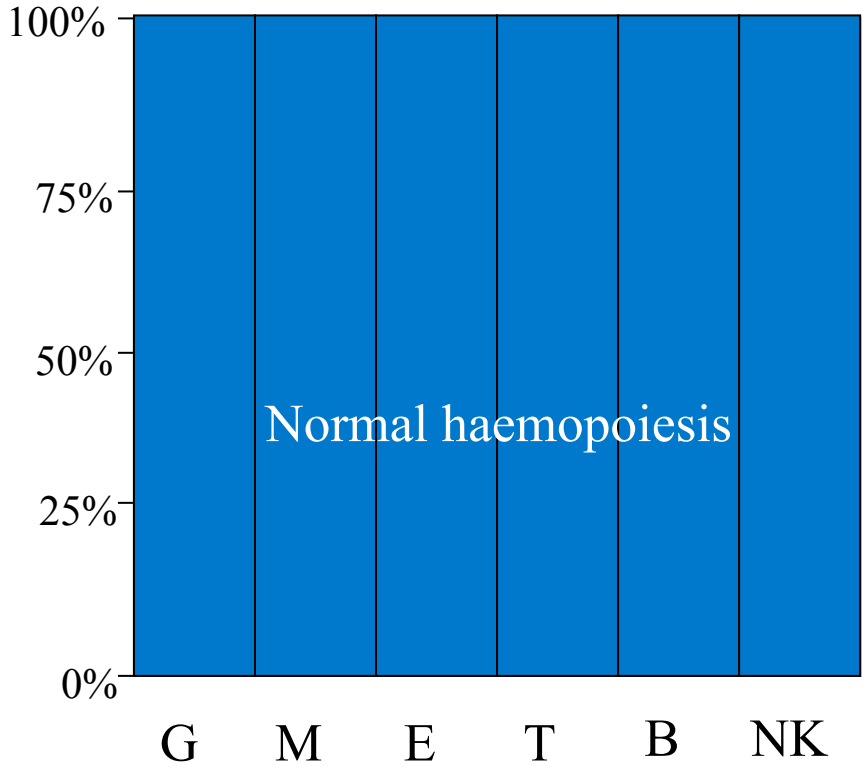




Bone Marrow Failure



Normal



The most widely accepted mechanism for clonal expansion of PNH-type cells in patients with BM failure is the “escape hypothesis” which states that the relative number of PIG-A mutant HSCs increases by avoiding immunological attacks by T cells.

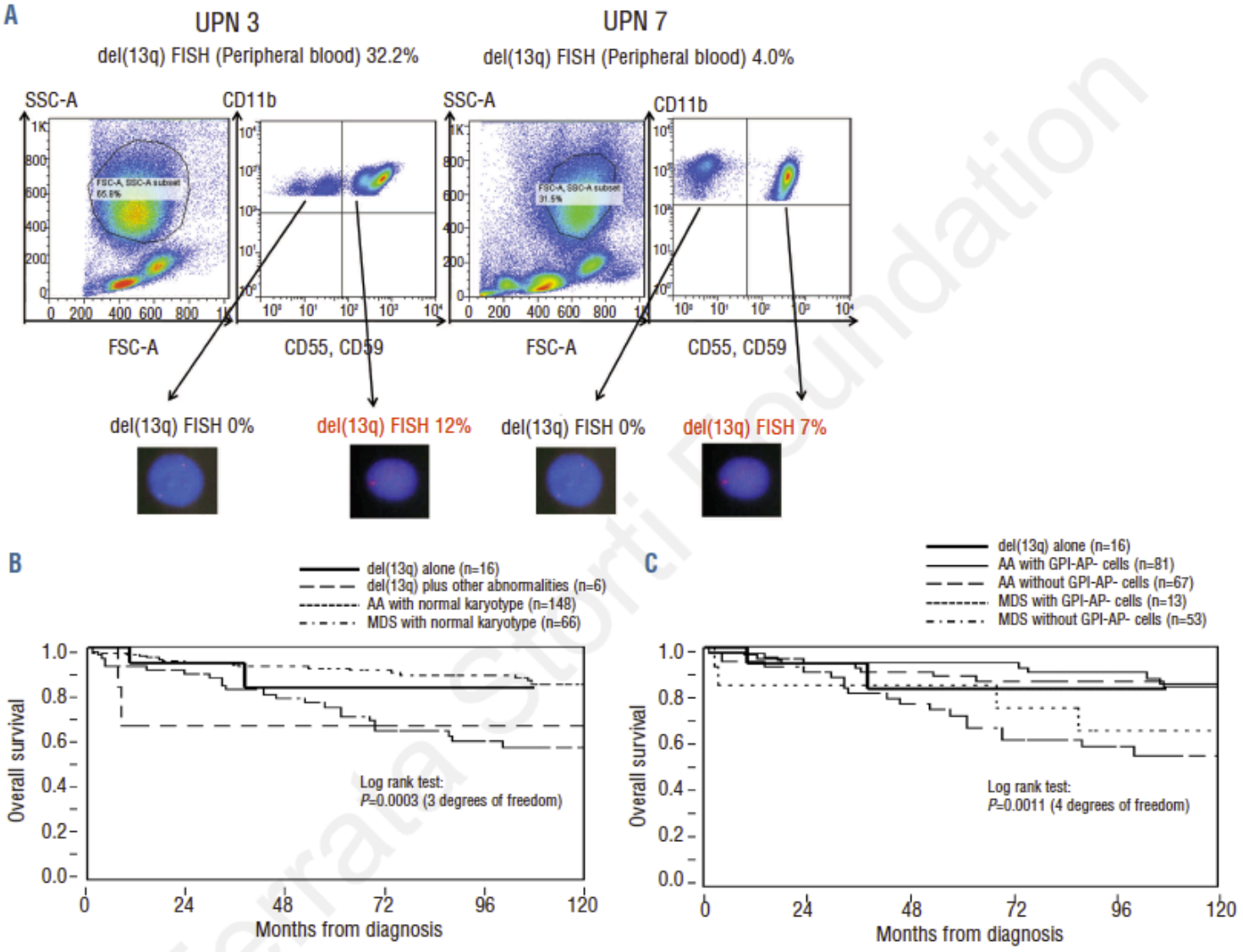
It should be further noted that the finding of such clones has not been related to clinical hemolysis, and specific PNH therapy is not indicated.

PNH Clone in MDS and AA

- **PNH cells (<5%) in 22% of 115 patients with AA and 23% of 39 with MDS; correlated with response to immunosuppression**
    - **Dunn DE, Ann Intern Med, 1999**

- **High incidence of an expanded PNH clone (<5%) in 136 patients with AA (32%) and MDS (18%); stable proportion over time**
    - **Maciejewski JP, Br J Haematol, 2001**

Del 13q



Hosokawa H et al, Haematologica | 2012; 97(12)

HLA Alleles

- HLA-DR15 (DR2) in 36% of 72 MDS and 42% of 59 AA
- In IBMTR, 30% of MDS and 33% of AA patients were HLA-DR2+
- HLA-DR4 has been reported to correlate with response to CyA in LGLL

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Somatic *STAT3* Mutations in Large Granular Lymphocytic Leukemia

40% pazienti T-LGLL (31/77)

blood

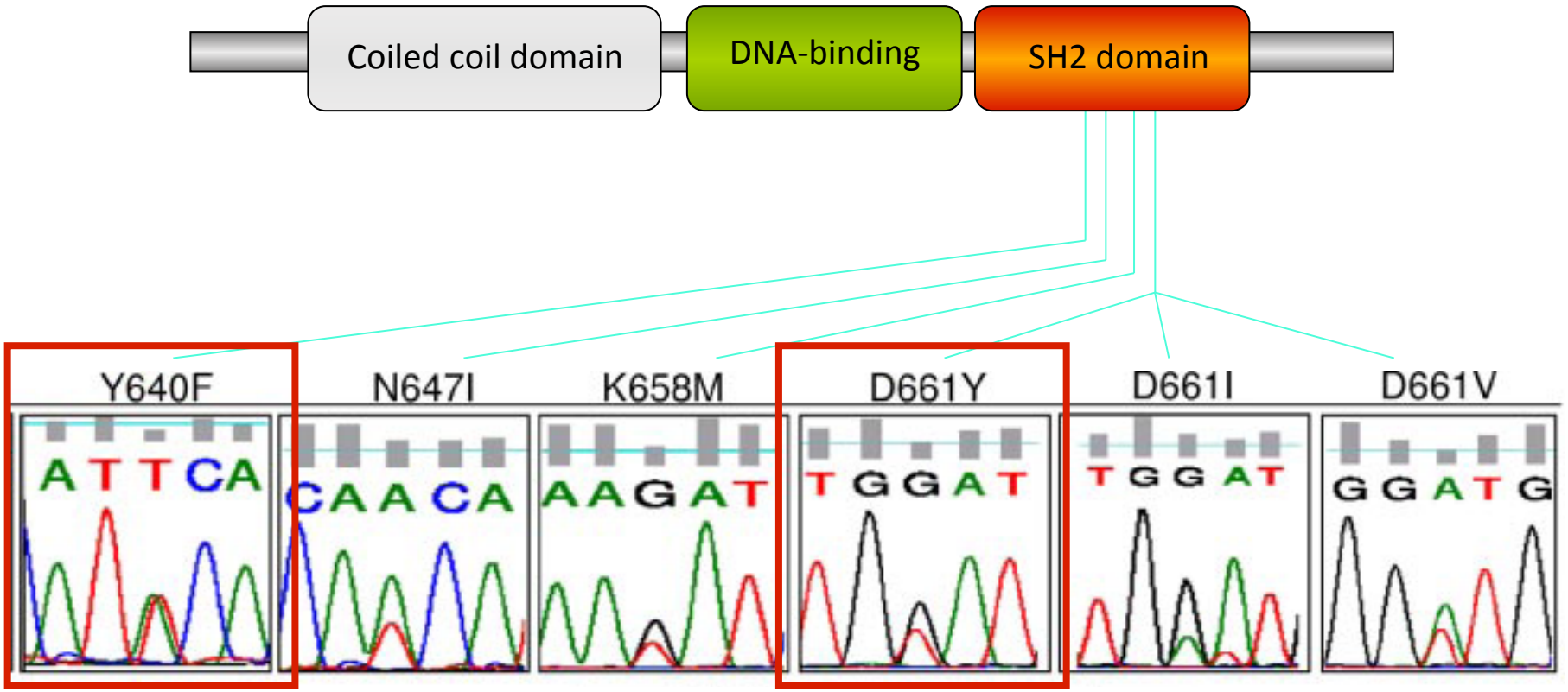
2012 120: 3048-3057
Prepublished online August 2, 2012;
doi:10.1182/blood-2012-06-435297

STAT3 mutations unify the pathogenesis of chronic lymphoproliferative disorders of NK cells and T-cell large granular lymphocyte leukemia

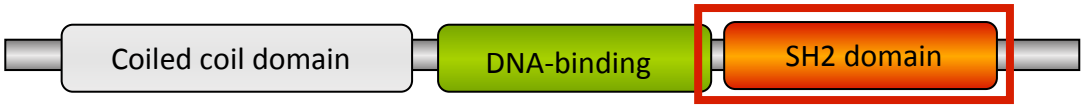
27% pazienti T-LGLL (33/120)
30% pazienti CLPD-NK (15/50)

MUTAZIONI DI STAT3 NELLE LGLL

- **mutazioni puntiformi missenso** nella regione SH2 del gene di **STAT3**
- **Le mutazioni** si ipotizza risultino in una maggiore stabilità dell'attivazione di STAT3
- Esistono lavori contrastanti su una possibile correlazione tra mutazioni di STAT3 e neutropenia

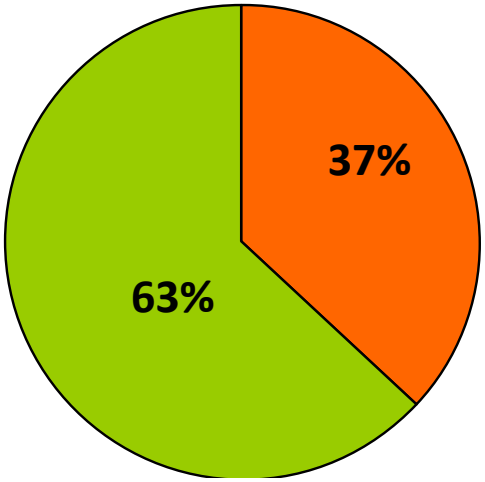


ANALISI DELLE MUTAZIONI DI STAT3



Exon 19-21

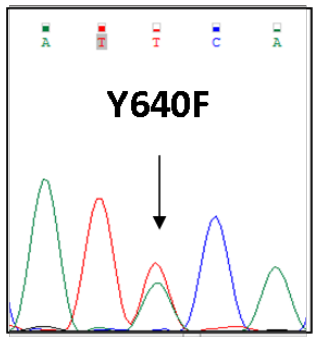
T-LGLL (n=101)



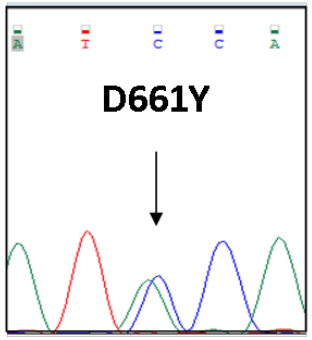
pz mutati

pz wild type

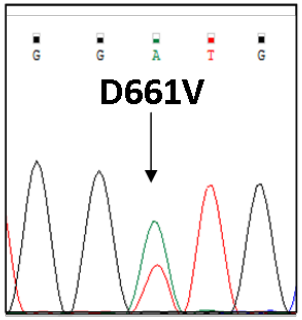
24 cases (65%)



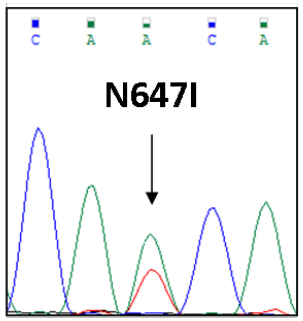
8 cases (22%)



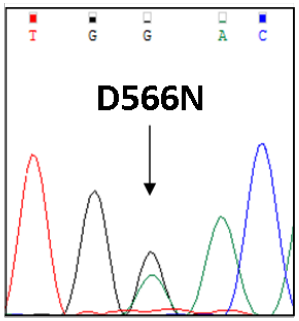
1 case (3%)



1 case (3%)



1 case (3%)



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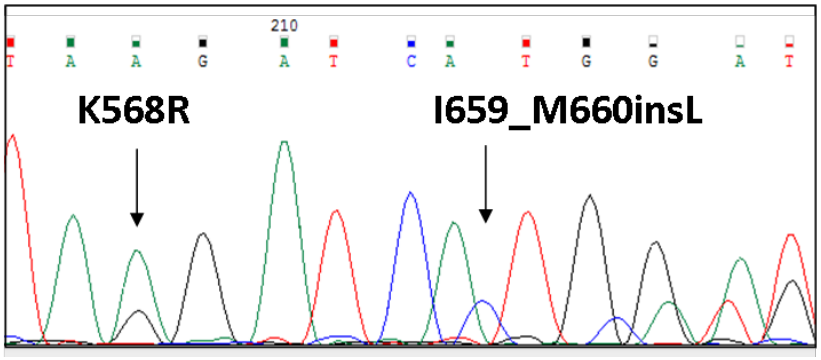
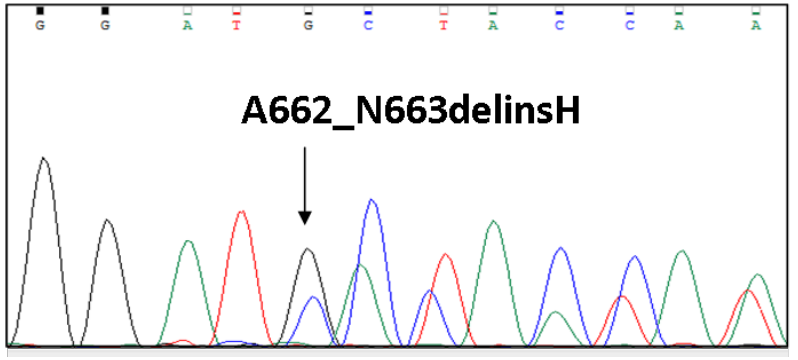
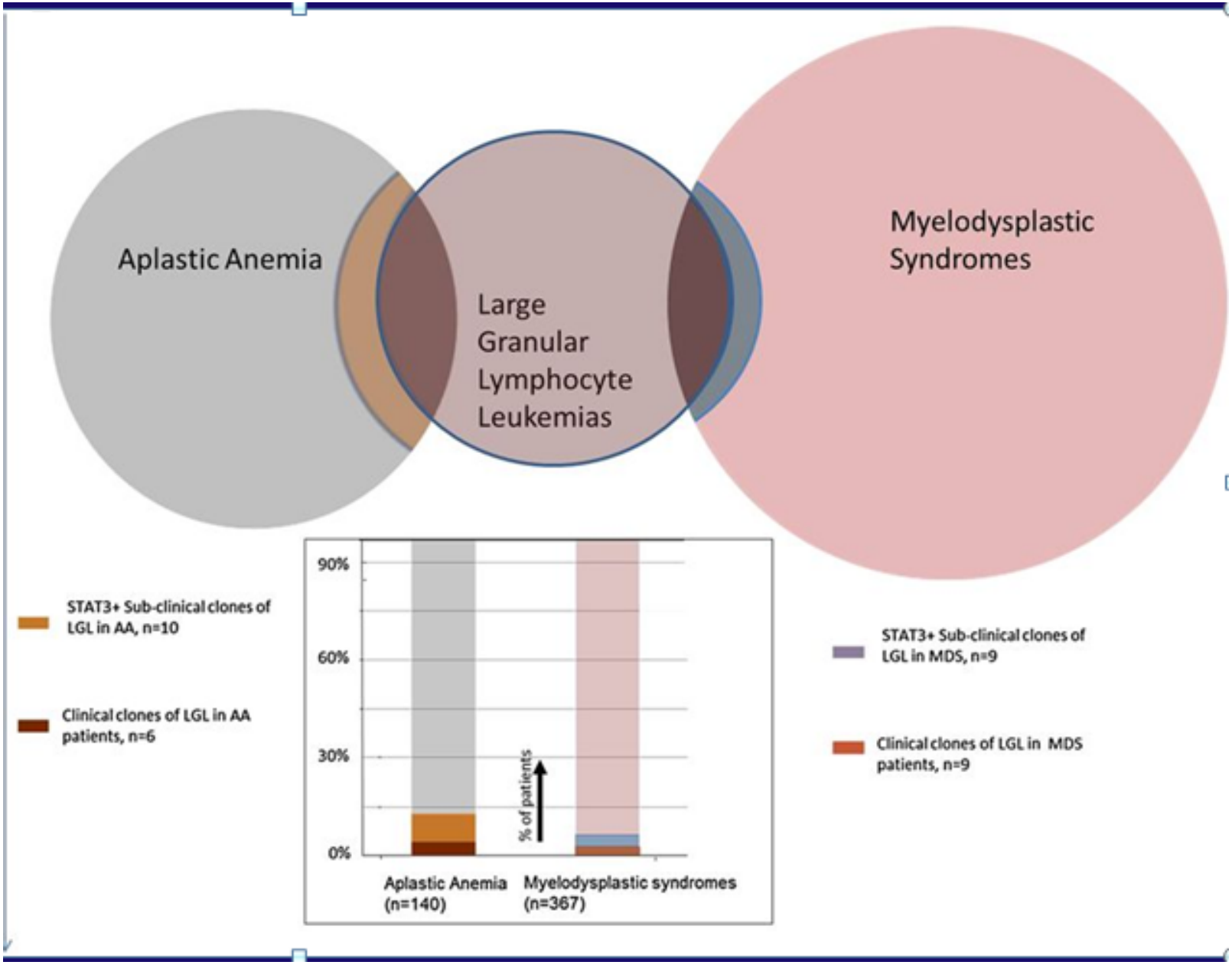


1 case (3%)



Clinically detected overlapping of LGL with AA and MDS



Jerez A et al. Blood 2013;122:2453-2459

Immunosuppressive therapy is effective in BMF

in particular:

- Anti-Thymocyte Globulin (ATG)
- Ciclosporin A

Conclusions

- Although an immune mediated mechanism is taking place in AA, MDS and LGL proliferations, these disorders show relevant differences

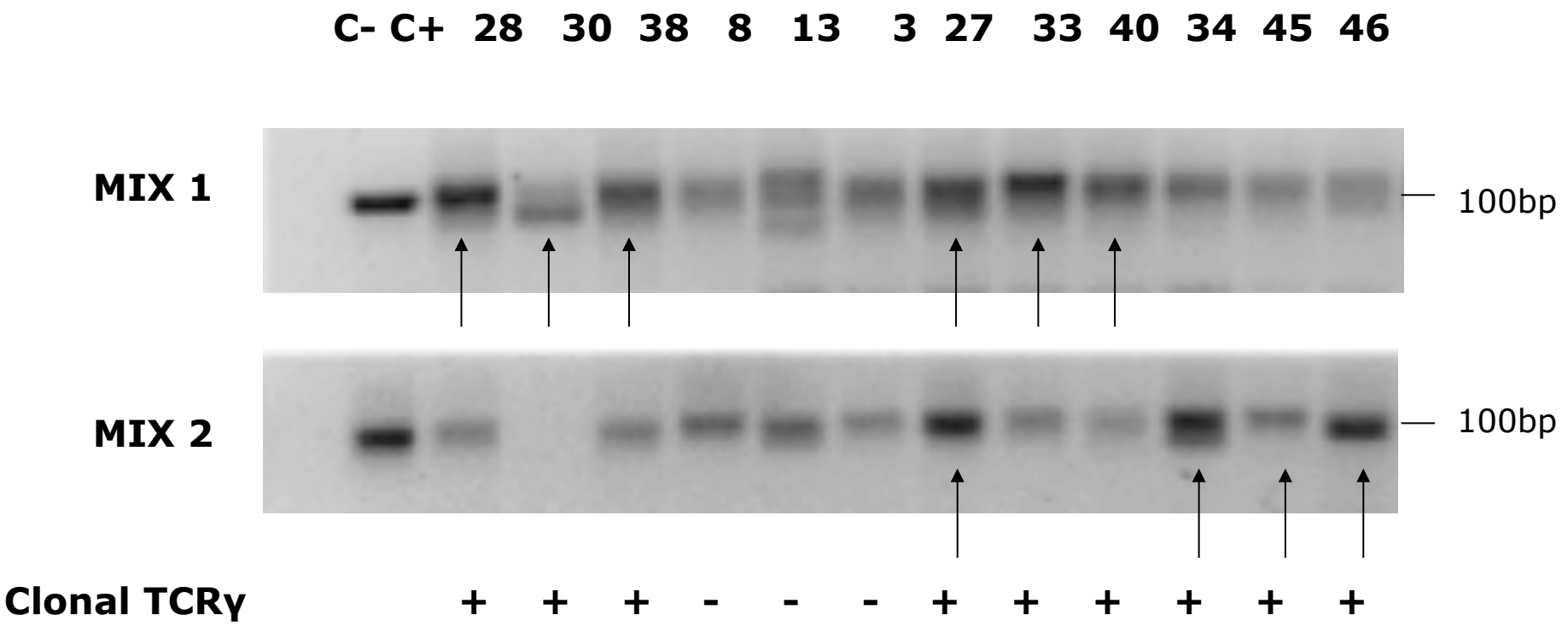
- Immortalization/ transformation via acquisition of a promoting somatic mutation in HPCs may be a mechanism contributing to immune-mediated BMF

If an antigenic pressure is present,

it should interest both

T cells and NK cells

TCRγ rearrangement analyzed by PCR in NK-CLPD patients (n:48)



One or two bands were detected in 23 out of 48 patients

Gattazzo et al Haematologica 2014

FONDAZIONE
ITALIANA
SINDROMI
MIELODISPLASTICHE

STUDIO DELLE CELLULE NATURAL KILLER IN PAZIENTI CON SINDROME MIELODISPLASTICA IPOCELLULATA

ID dello studio: FISM-hMDS14

Versione n° 1- 20 novembre 2014

Sponsor dello studio:
Fondazione Italiana Sindromi Mielodisplastiche Onlus (FISM)

Coordinatore:
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Fax: 0131 263455
Mail: segreteriafismonlus@ospedale.al.it
Sito web: www.fismonlus.it

Obiettivi dello studio

- **studiare la rappresentazione dei *subset* NK nel midollo e nel sangue periferico di pazienti con hMDS, in particolare la percentuale di NK $CD56^{bright}/CD16^{low}$, dotate di maggiore capacità di secrezione citochinica e la percentuale di cellule $CD56^{low}/CD16^{bright}$**

- **valutare le mutazioni di STAT3 nelle cellule derivanti da sangue midollare e periferico di pazienti affetti da MDS ipocellulate e valutarne il ruolo. Particolare attenzione sarà dedicata a capire se le mutazioni interessino più tipi cellulari o le cellule NK**

- **Analisi dell'aplotipo KIR, dell'espressione dei KIR e della metilazione del loro promotore**