FONDAZIONE ITALIANA SINDROMI MIELODISPLASTICHE



CONVEGNO FISiM

Firenze, CSF Montedomini
"Il Fuligno"
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MDS e germline predisposition

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Disclosures of Enrico Attardi

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
Ascentage						x	
AbbVie					x		

Myeloid Neoplasms with germline predisposition

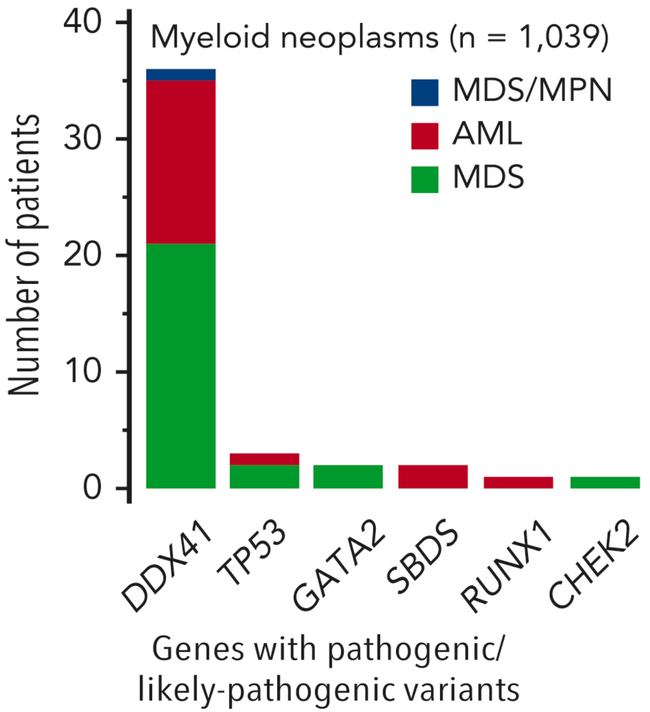
WHO 2022		ICC
Myeloid neoplasms with germline predisposition without a pre-existing platelet disorder or organ dysfunction		**Hematologic neoplasms with germline predisposition without a constitutional disorder affecting multiple organ systems**
Germline *CEBPA* P/LP variant (CEBPA-associated familial AML)		Myeloid neoplasms with germline *CEBPA* mutation
Germline *DDX41* P/LP variant *(myeloid and lymphoid neoplasms)*		Myeloid or lymphoid neoplasms with germline *DDX41* mutation
Germline *TP53* P/LP variant (Li–Fraumeni syndrome) *(myeloid and lymphoid neoplasms)*		Myeloid or lymphoid neoplasms with germline *TP53* mutation
Myeloid neoplasms with germline predisposition and pre-existing platelet disorder		**Hematologic neoplasms with germline predisposition associated with a constitutional platelet disorder**
Germline *RUNX1* P/LP variant (familial platelet disorder with associated myeloid malignancy, FPD-MM) *(myeloid and lymphoid neoplasms)*		Myeloid or lymphoid neoplasms with germline *RUNX1* mutation
Germline *ANKRD26* P/LP variant (thrombocytopenia 2) *(myeloid and lymphoid neoplasms)*		Myeloid neoplasms with germline *ANKRD26* mutation
Germline *ETV6* P/LP variant (thrombocytopenia 5) *(myeloid and lymphoid neoplasms)*		Myeloid or lymphoid neoplasms with germline *ETV6* mutation
Myeloid neoplasms with germline predisposition and potential organ dysfunction		**Hematologic neoplasms with germline predisposition associated with a constitutional disorder affecting multiple organ systems**
Germline *GATA2* P/LP variant (GATA2-deficiency)		Myeloid neoplasms with germline *GATA2* mutation
Germline *SAMD9* P/LP variant (MIRAGE syndrome)		Myeloid neoplasms with germline *SAMD9* mutation
Germline *SAMD9L* P/LP variant (SAMD9L-related Ataxia-Pancytopenia syndrome		Myeloid neoplasms with germline *SAMD9L* mutation
Bone marrow failure syndromes		Myeloid neoplasms associated with bone marrow failure syndromes
Severe congenital neutropenia (SCN)		Severe congenital neutropenia
Fanconi anemia (FA)		Fanconi anemia
Shwachman–Diamond syndrome (SDS)		Shwachman–Diamond syndrome
Telomere biology disorders		Telomere biology disorders including dyskeratosis congenita
		Diamond–Blackfan anemia
RASopathies (Neurofibromatosis type 1, CBL syndrome, Noonan syndrome or Noonan-like disorders) *(myeloid and lymphoid neoplasms)*		JMML associated with neurofibromatosis JMML associated with Noonan syndrome-like disorder (CBL syndrome)
Down syndrome		Myeloid or lymphoid neoplasms associated with Down syndrome
Biallelic germline *BLM* P/LP variant (Bloom syndrome)		

Similarity / Overlap between the WHO (2022) and ICC

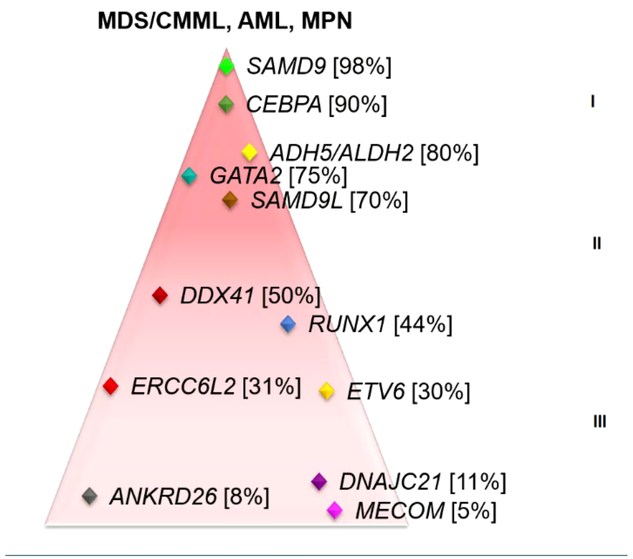
- Very strong overlap or Identical categories
- Slight differences between the two classification system
- Considerable difference between the two classification systems

Characteristics of Germline Myeloid Neoplasms/BMF

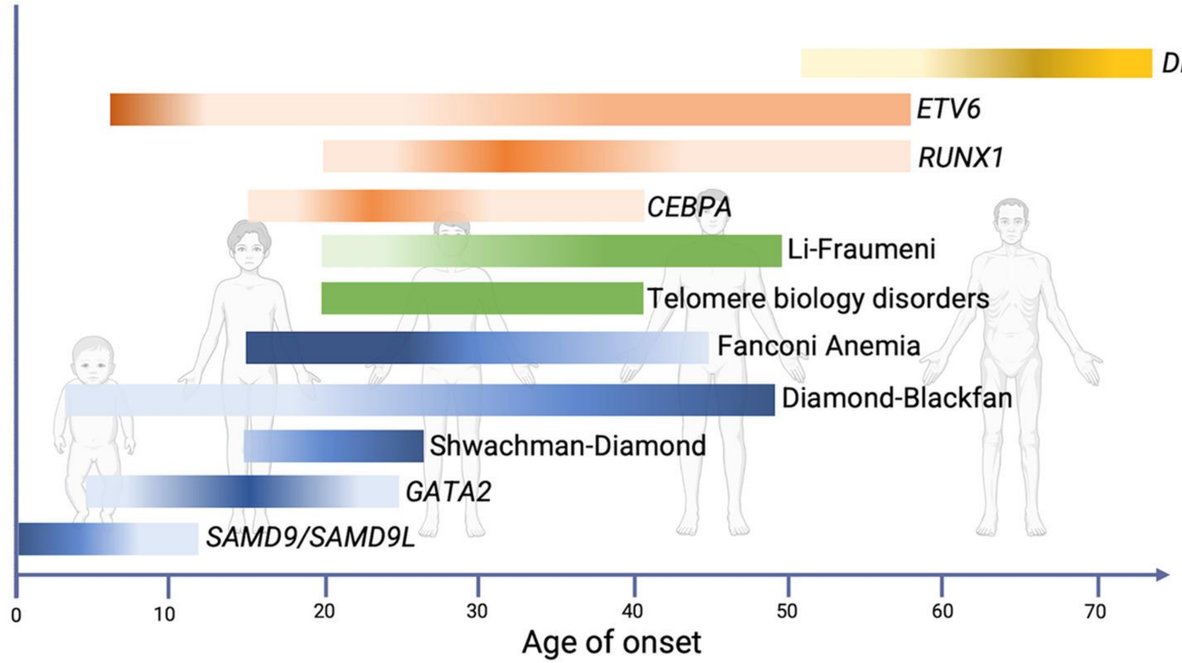
GL predisposition genes in MN



Penetrance



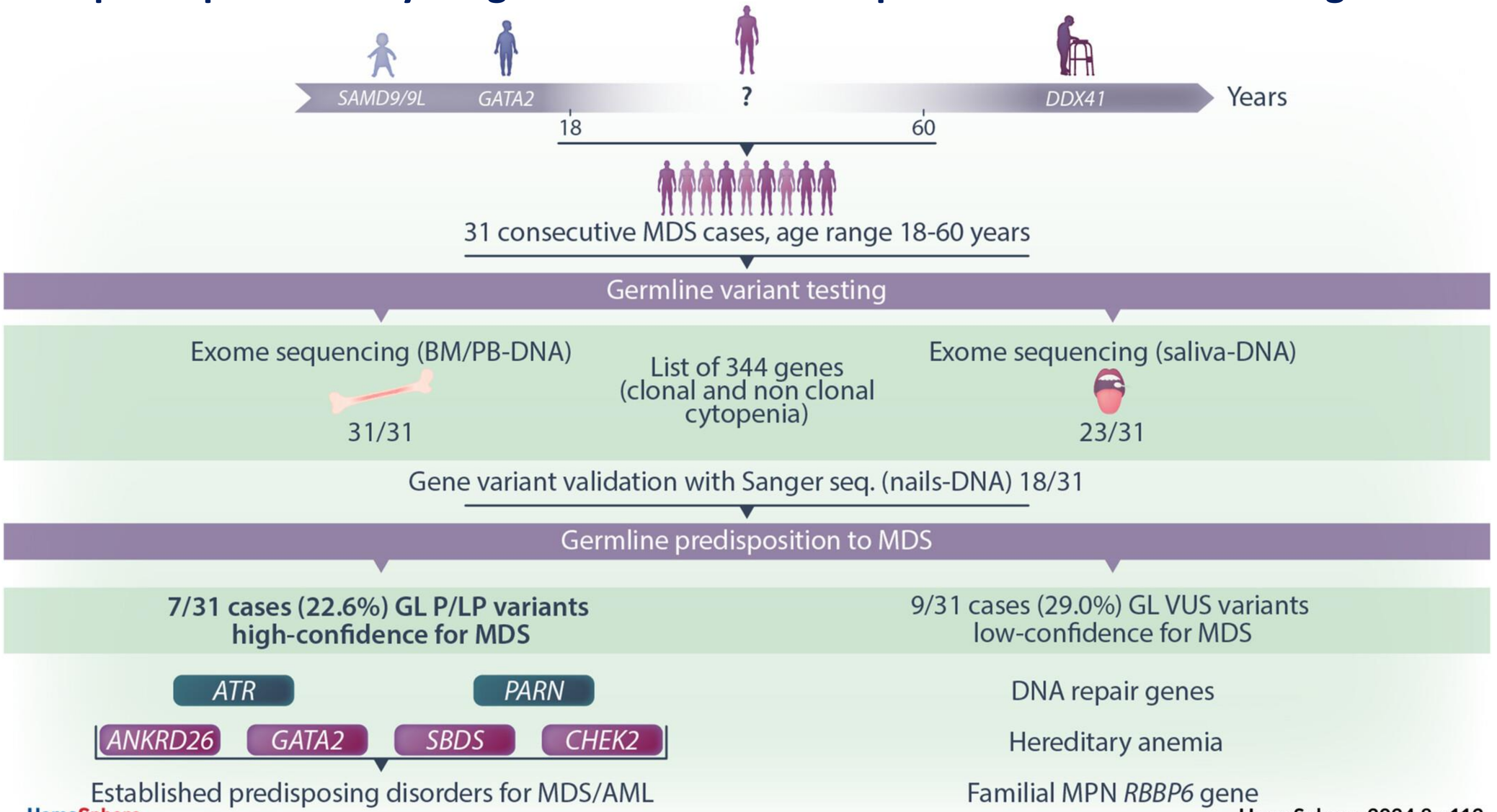
Age-prevalence



Estimated frequency: 5-15% of adult MDS

Makishima et al, Blood 2022
Feurstein S., EHA 2023
By courtesy of Wlodarski M.

Germline predisposition in younger MDS cases: the importance of a scalable diagnostic model



HemaSphere

HemaSphere. 2024;8:e112
Attardi E, Santini V et al

Progetto GEMMA Genetica-Ematologia

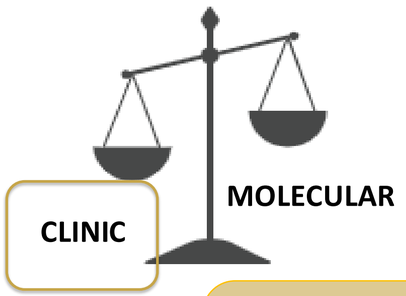
Checklist for patient selection

	Eligibility criteria (at least one is required)	YES	NO
Familial history	Family history of at least **one first- or second-degree** relative** affected by BMF/ICUS (or CCUS)/myeloid neoplasm (MN)		
	Family history of at least 2 first- or second-degree relatives affected by an oncologic/hematologic condition (**including solid tumors**)		
	Family history of at least **3 relatives (regardless of degree)** affected by an oncologic/hematologic condition (including solid tumors)		
Personal history	Personal history of at least **two cancers**, one of which is a myeloid neoplasm		
	Extra-hematological signs/symptoms suggestive of a syndromic condition If yes, specify		
Disease characteristics	MDS with age at **onset <50 years**		
	ICUS/CCUS with age at onset <50 years and **bone marrow biopsy showing <30%** cellularity (or age-adjusted hypocellularity)		
	Detection of gene mutation with VAF ≈ 50% (or 100%) and known as germline, using NGS analysis		

* For myeloid neoplasm (MN), refer to one of the following: MDS, AML, MPN, CML, or CMML.

** First-degree relatives: children and parents (direct line). Second-degree relatives: siblings (collateral line), grandchildren and grandparents (direct line).

Algorithm of the Hematology-Genetics outpatient clinic (Progetto GEMMA)



Hematology-Genetics outpatient clinic «GEMMA»

Are skin fibroblasts available?

yes

Clinical exome sequencing
DNA from skin fibroblasts

Diagnosis of BMF/LPS

no

(i.e. severe thrombocytopenia)

Clinical exome sequencing
DNA from CD3+ or BM/PB mononuclear cells

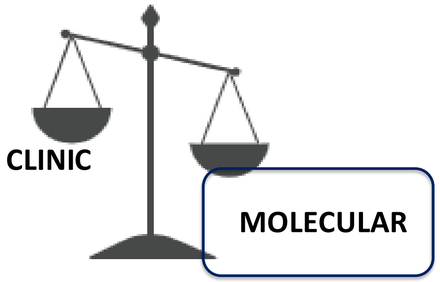
LP/P variant in BMF/LPS genes

Sanger sequencing
(e.g DNA from nails)
Confirming germline variants in probands

Sanger sequencing
DNA from PB mononuclear cells
Confirming the inheritance

- BM, Bone Marrow
- BMF, Bone Marrow Failure syndromes
- LPS, Leukemia Predisposition Syndromes
- P, Pathogenic
- PB, Peripheral Blood

Algorithm of the Hematology-Genetics outpatient clinic (Progetto GEMMA)



NGS myeloid panel
in clinical routine
DNA from BM/PB
mononuclear cells

~50% VAF in genes known to be involved
in BMF/LPS

→ *Sanger sequencing*
DNA from nails/CD3+ cells
Confirming germline variants

VUS with ~50% VAF
in genes associated
(as somatic) to clonality
in hematological malignancies

→ *Sanger sequencing*
DNA from nails/CD3+ cells
Excluding somatic mutations

- BM, Bone Marrow
- NGS, Next Generation Sequencing
- PB, Peripheral Blood
- VAF, Variant Allele Frequency
- VUS, Variant of Uncertain Significance

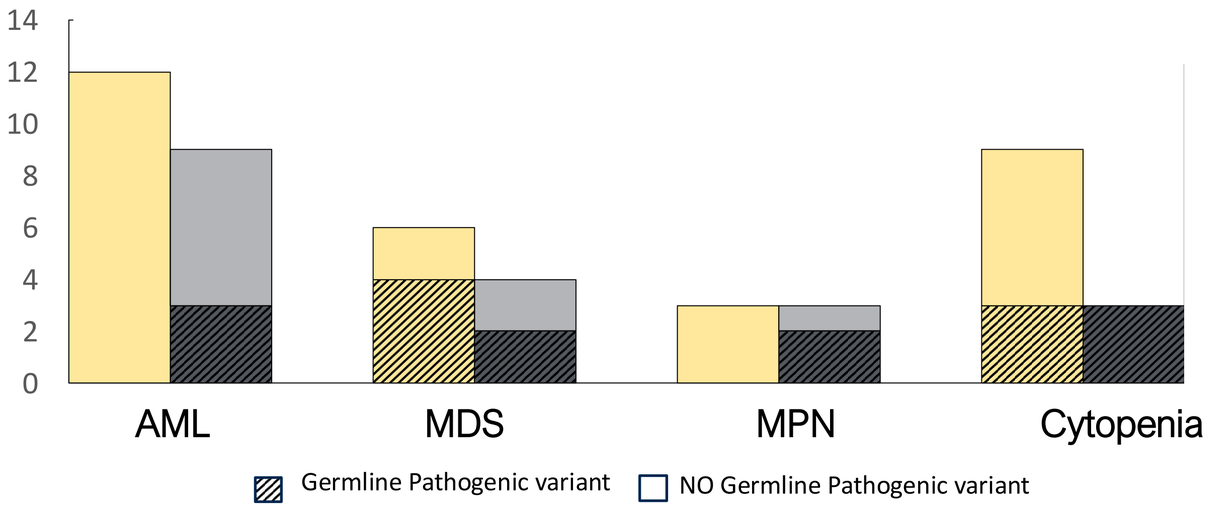
GEMMA Project (years 2023-26)

Total n = 107

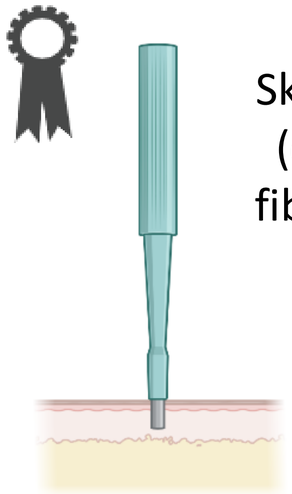
- 55 patients
- 52 family members

Exome sequencing targeting of 330 genes/WES
n=31 pts

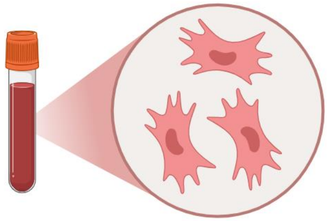
Germline variant validation through Sanger sequencing after NGS
n=24 pts



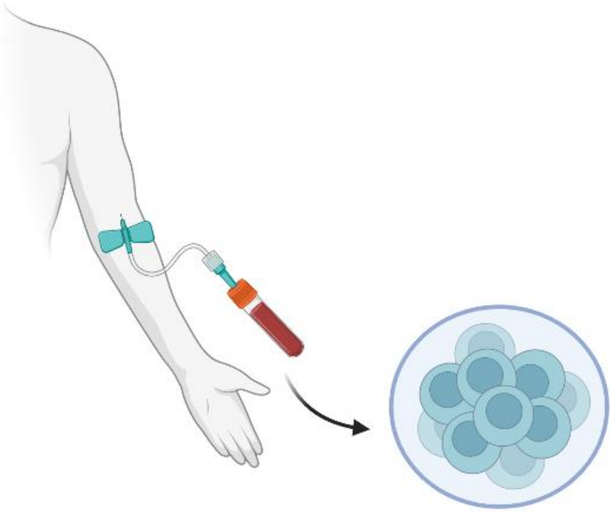
Genetic diagnoses	Probands	Specimen	family members
	N = 17/55 (30,9%)		N = 6/52 (11,5%)
SH2B3/LNK mutated MPN	2	CD3+(2)	2
GATA2 deficiency	4	CD3+(4) LMC(1)	2
Myeloid neoplasms with DDX41	8	Nails (5) CD3+(3)	2
Telomere biology disorders (TERC)	-		1
Fanconi Anemia (FANCA)	1	Fibrobl. (1)	-
*RUNX1 familial platelet disorder	2	Nails (1) CD3+(1)	-



Skin biopsy (cultured fibroblasts)

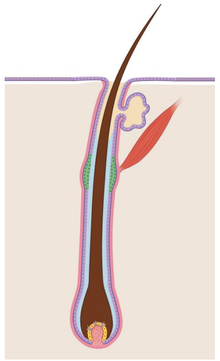


BM-derived mesenchymal stromalcells

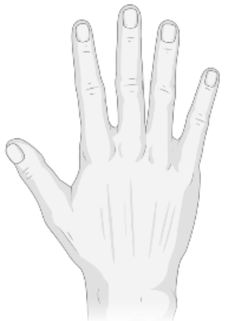


Lymphocytes CD3+

What is the best specimen for germline testing?

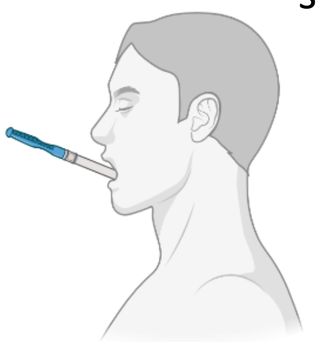


Hair follicles



Nails

Saliva/Buccal swab



1. Clinical case

A 33-year-old male patient

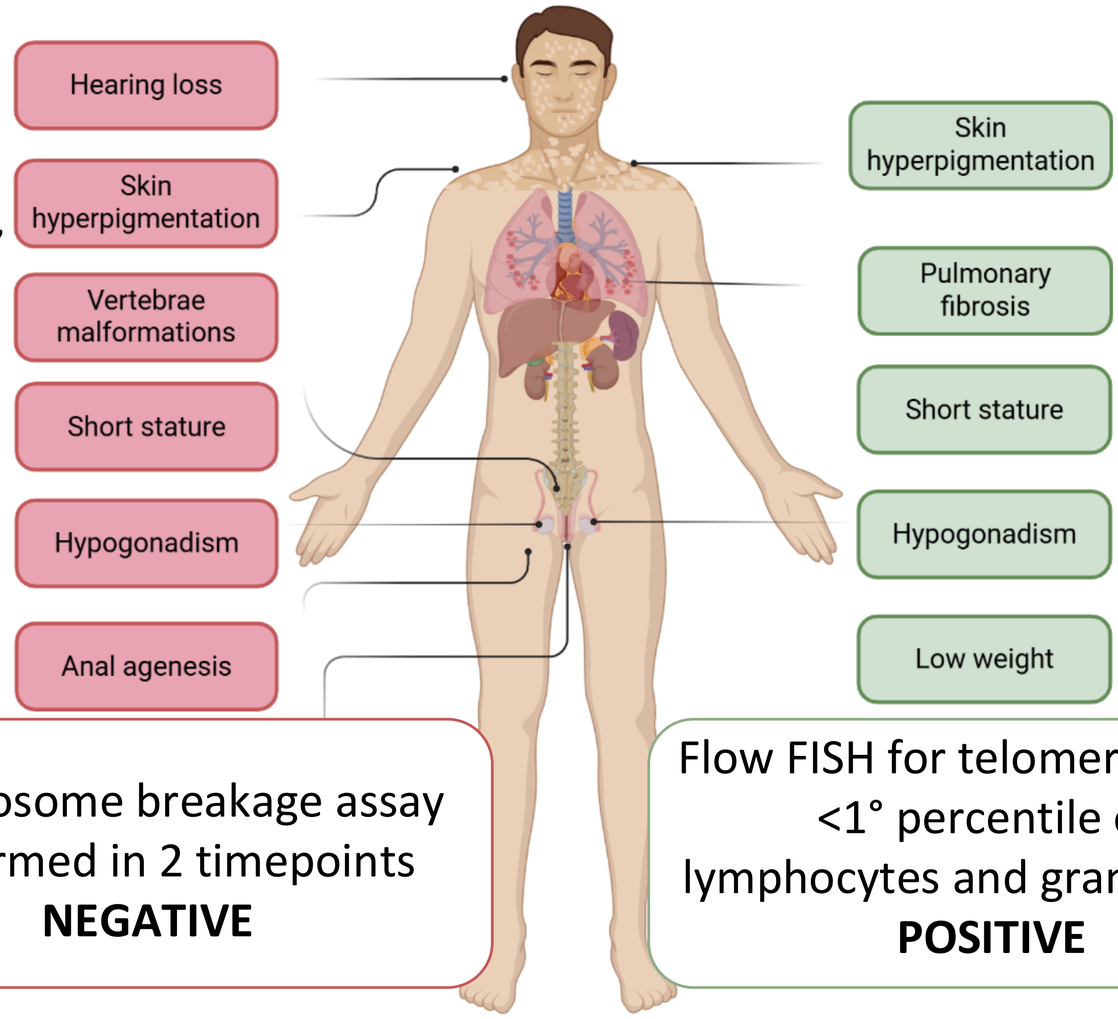
Ospedale S. Spirito Roma, ASL RM1

- **Onset**: pancytopenia.
- **Blood count at the onset**: Hb 7.7 g/dl, MCV 108 fL, WBCs 2.300/mm^3, ANC 760/mm^3, plts 26.000/mm^3.

⇩

Bone marrow needle aspiration and biopsy:
Significant dysplasia of the erythroid lineage with maturation defect;
Marked hypocellularity (blasts <5%);
Hypoplastic MDS?

Fanconi Anemia (FA) **vs.** Telomere Biology Disorders (TBD)

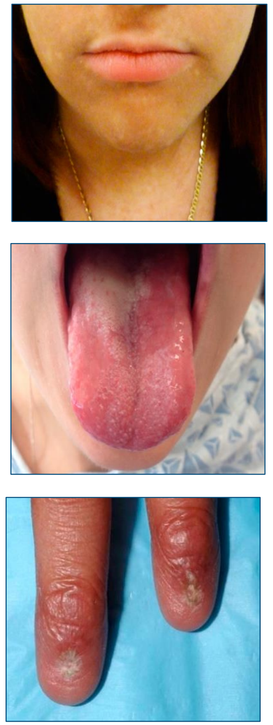


Chromosome breakage assay performed in 2 timepoints
NEGATIVE

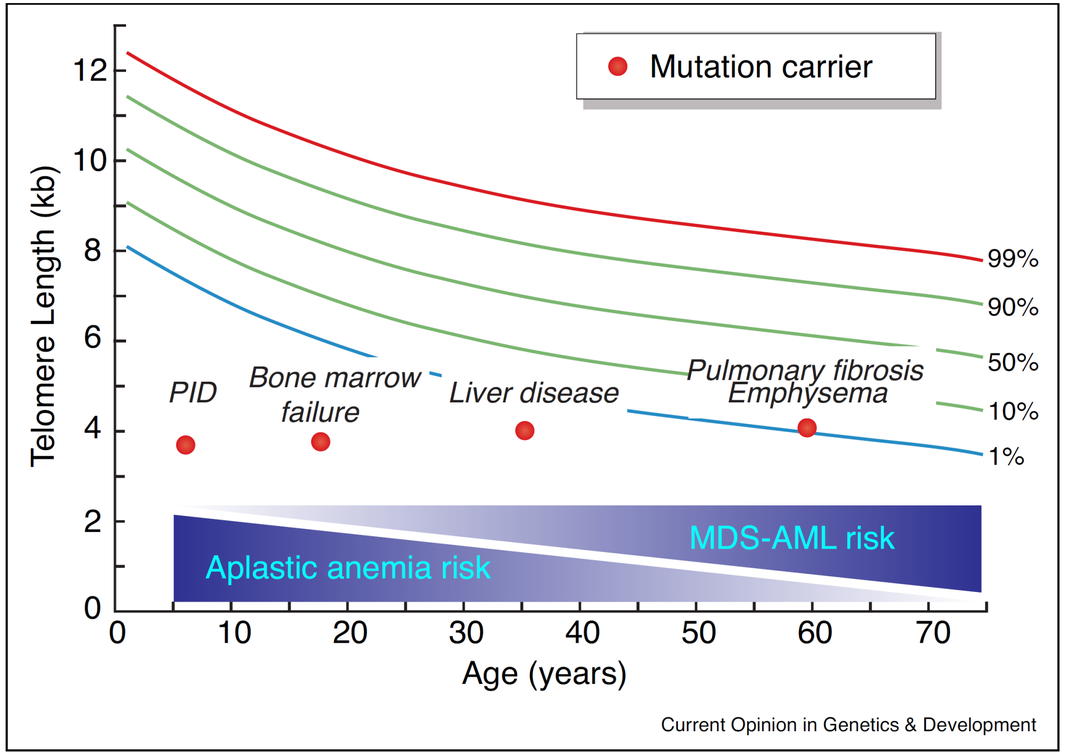
Flow FISH for telomeres length <1° percentile on lymphocytes and granulocytes
POSITIVE

Telomere Biology Disorders

Known DC/TBD-associated genes
(*ACD, CTC1, DKC1, NAF1, NHP2, NOP10, PARN, POT1, RTEL1, STN1, TERC, TERT, TINF2, WRAP53, ZCCHC8*).
Other genes to consider, recently discovered: *RPA1, DCLRE1B, MDM4*.



classical triad of dyskeratosis congenita



>99%: *POT1* tumor predisposition syndrome

1-10%: TBD

Flow-FISH

Savage SA. Dyskeratosis congenita and telomere biology disorders. Hematology Am Soc Hematol Educ Program. 2022;
Schratz KE, Armanios M. Cancer and myeloid clonal evolution in the short telomere syndromes. Curr Opin Genet Dev. 2020.

WES analysis

whole exome sequencing analysis:

2 compound heterozygous variants in *FANCA*

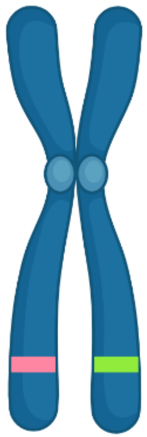
- c.2678G>A, p.W893*
- 189+1G>A

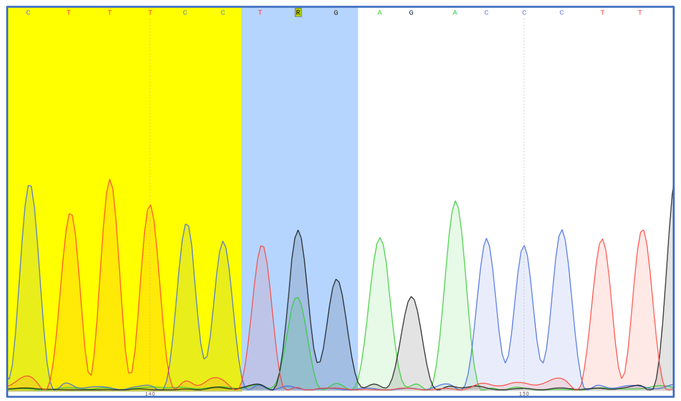
A challenging Diagnosis

Sanger analysis performed on **CD3+** isolated from peripheral blood **Fibroblasts** isolated and cultured from skin biopsy

FIBROBLASTS

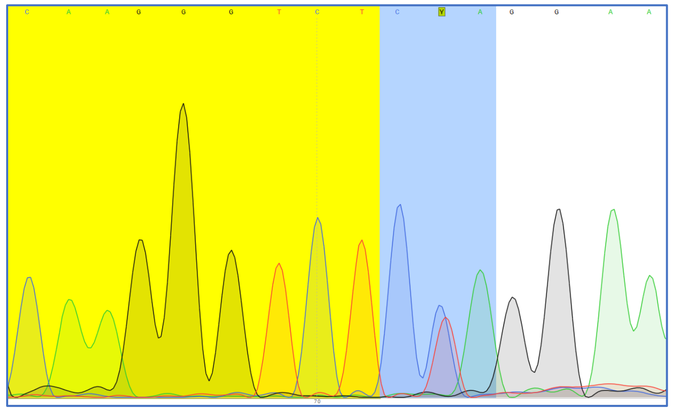
FANCA c.2678G>A p.W893*





Forward

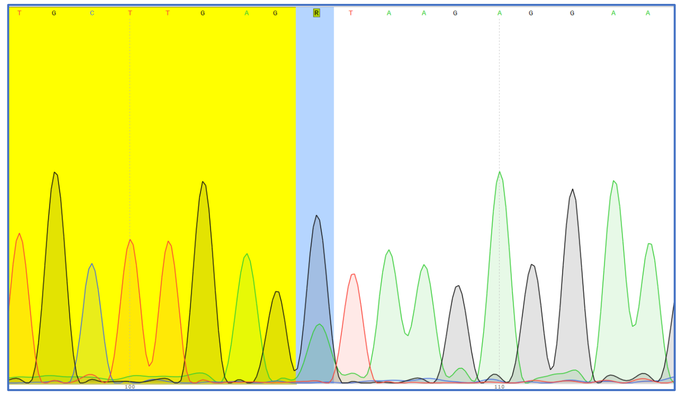
5’ – TGG – 3’ > 5’ – TAG – 3’



Reverse

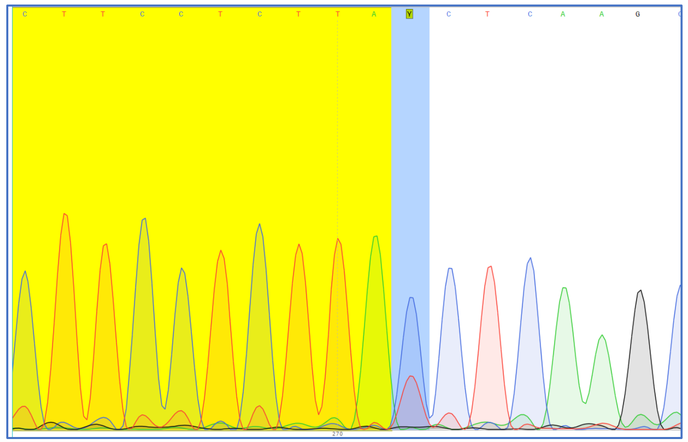
5’ – CCA – 3’ > 5’ – CTA – 3’

FANCA c.189+1G>A



Forward

5’ –GTA – 3’ > 5’ –ATA – 3’



Reverse

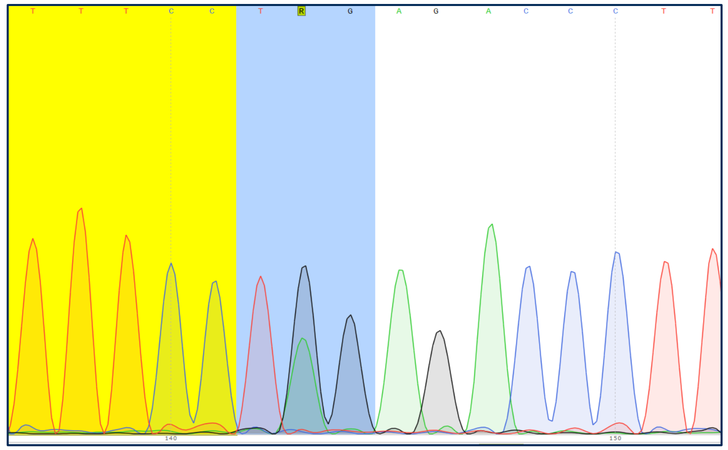
5’ – TAC – 3’ > 5’ – TAT – 3’

A challenging Diagnosis

CD3+ cells

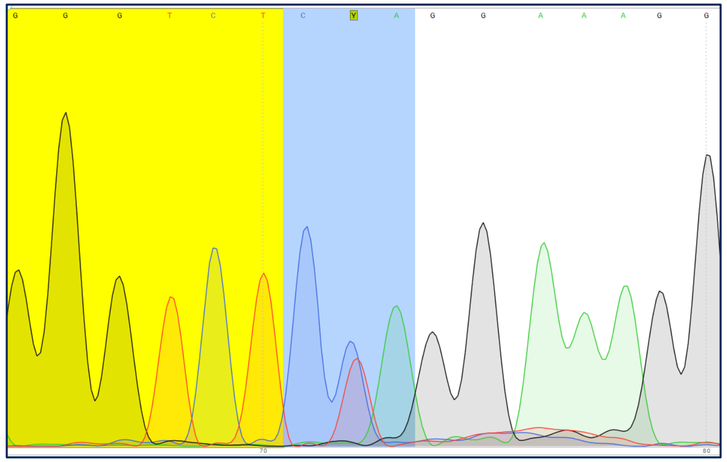
FANCA* c.2678G>A p.W893





Forward

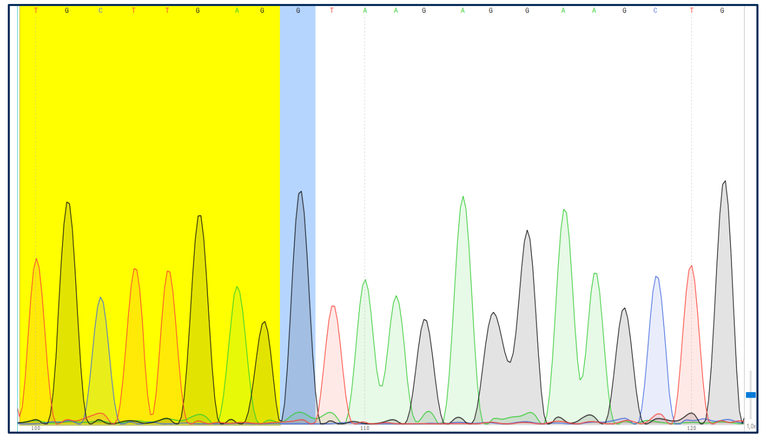
5’ – TGG – 3’ > 5’ – TAG – 3’



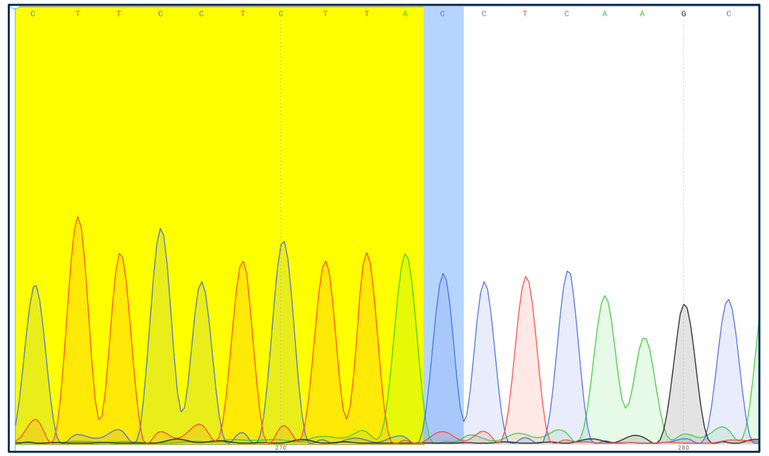
Reverse

5’ – CCA – 3’ > 5’ – CTA – 3’

Absence of *FANCA* c.189+1G>A (wild type)



Forward

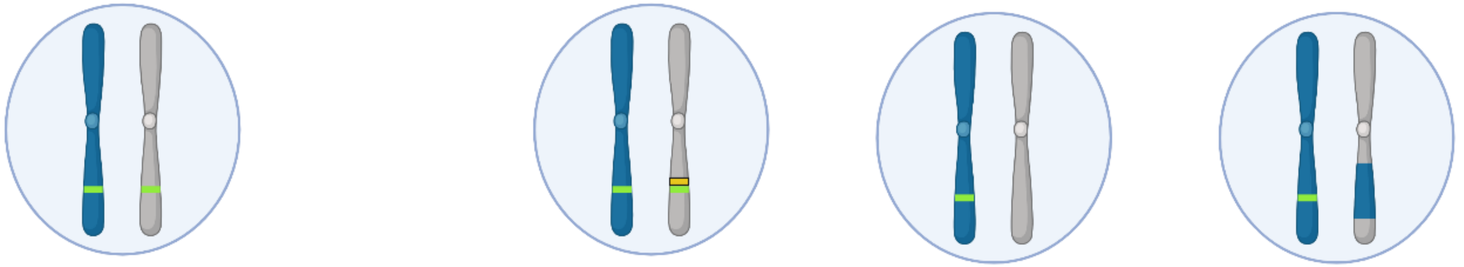


Reverse

Somatic genetic rescue

Somatic rescuing in FA

Restricted cell fitness → **Functional hematopoiesis**



Fanconi anemia germline *FANCA*, *FANCC* or *FANCD2*

second-site variant

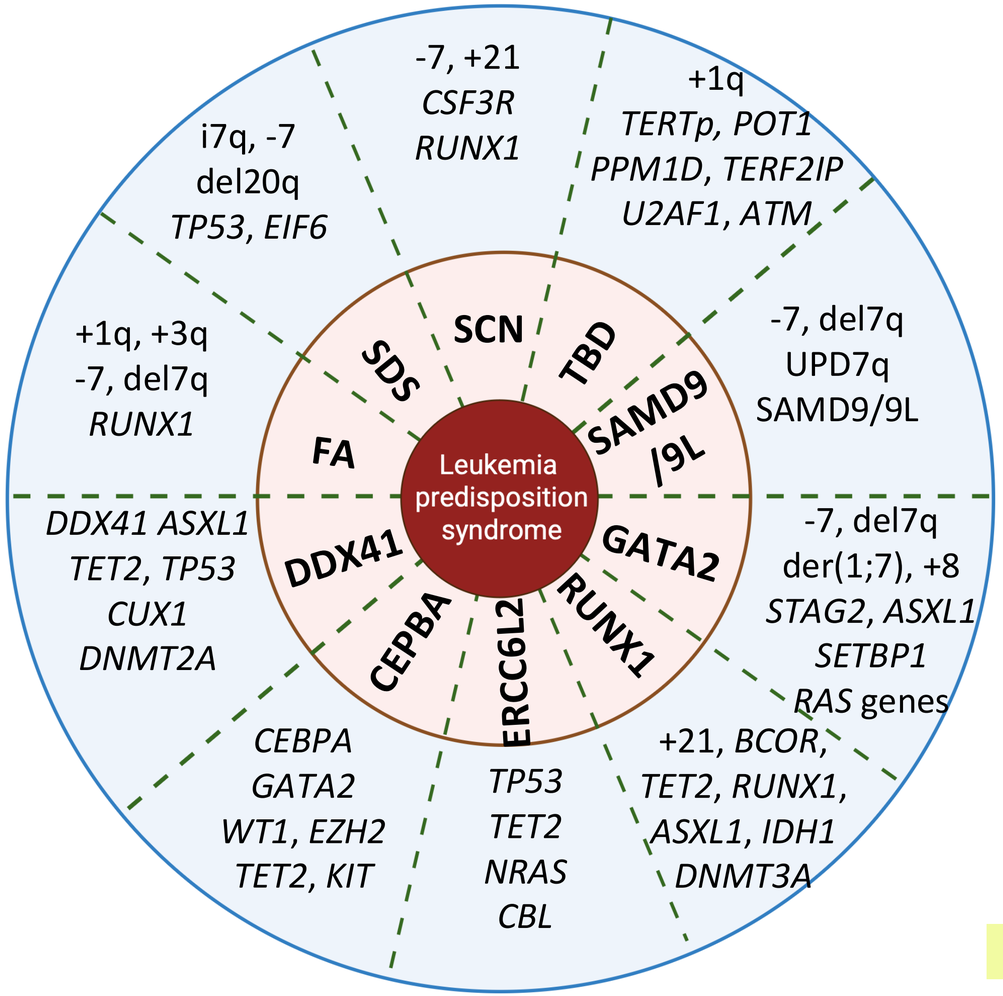
back mutation

intragenic recombination

Somatic rescuing in lymphocytes explains the negative chromosome breakage assay

Clonal trajectories in MDS with germline predisposition

CH-IPI:
clonal hematopoiesis in predisposed individuals



Important for:

1. Diagnosis (red flags)
2. Disease confirmation
3. Disease monitoring (MDS/AML)

2. Clinical case

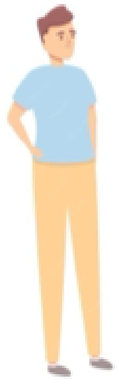
2014



69 yo male, with mild pancytopenia > 6 mos
- PMH: hypertension, surgery for gastric ulcer, pacemaker
- BM: trilinear dysplasia, 2% blasts, decreased megakaryocytes
- Conventional karyotyping: 46,XY[20]
- BOM: hypocellular (20%). Monocyte hyperplasia (10%), Blasts < 5%.
Diagnosis: LR-MDS (IPSS-R: 3)

2018,
73 yrs

Disease progression to HR-MDS (IPSS-R: 5.5)
- NGS myeloid panel: *DNMT3A* (28%)
- Start azacitidine

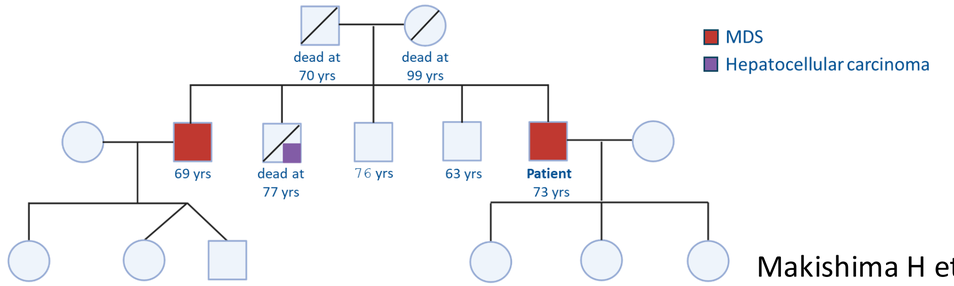


Younger brother, 67 yrs
- HR-MDS, 12% blasts,NK
(WHO 2022: MDS-IB1)
Start azacitidine

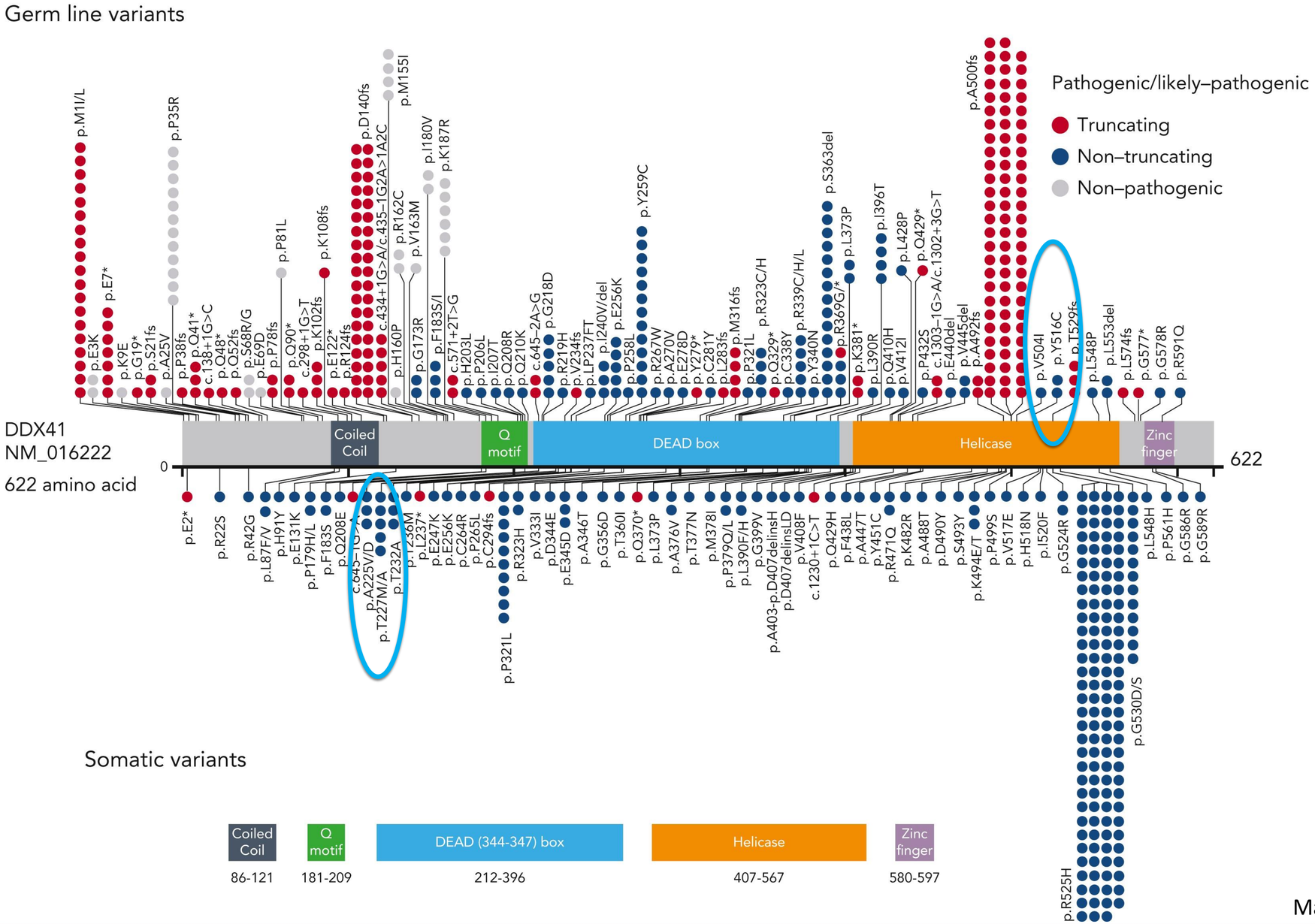
NGS: *DDX41*:p.Tyr516Cys, (VAF 48%)
& *DDX41*:p.Thr227Met,(VAF 10%)

→ family's pedigree

DDX41:p.Tyr516Cys, (VAF 53%)



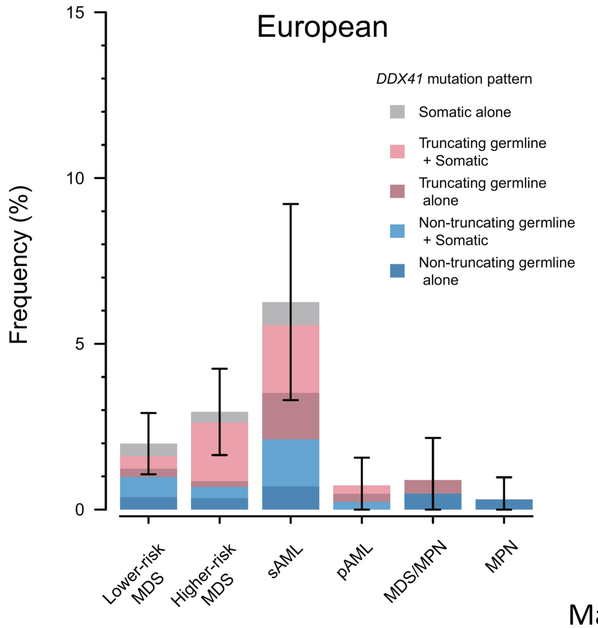
Makishima H et al. s, Blood 2023

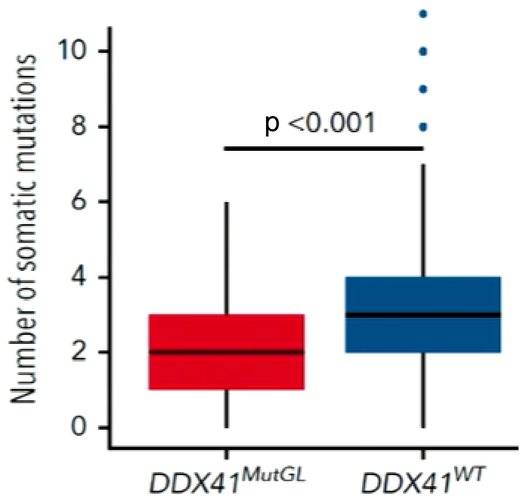


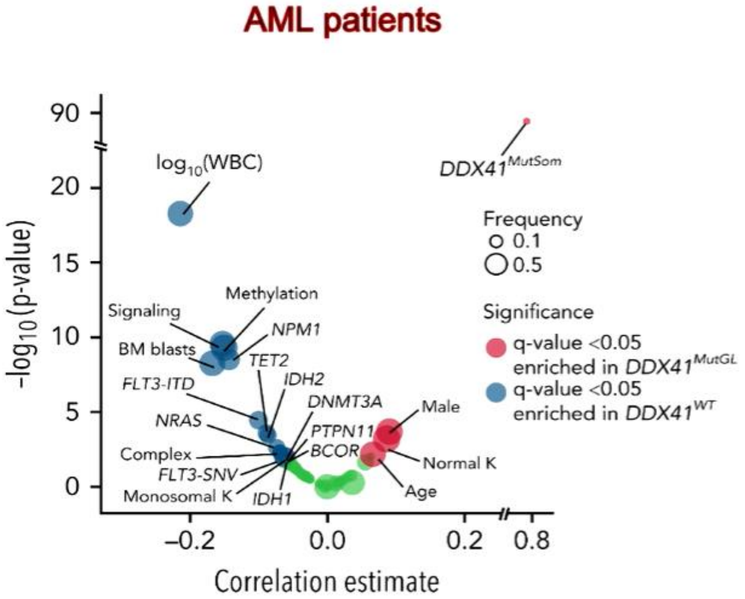
Makishima H et al. s, Blood 2023

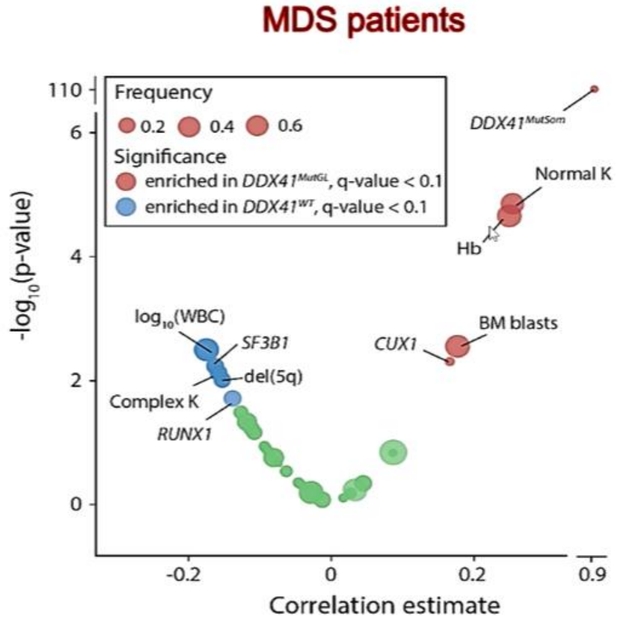
DDX41 germline predisposition

- Transmission autosomal dominant, represents about 5% of all myeloid malignancies
- DDX41 is critical for ribosome biogenesis through small nucleolar RNA processing (snoRNA);
- Biallelic *DDX41* mutant BM cells exhibit impaired snoRNA processing and ribosome function, which leads to cell death;
- An increased inflammatory status is common;
- DDX41mut adult MDS/AML, median age 69 years.



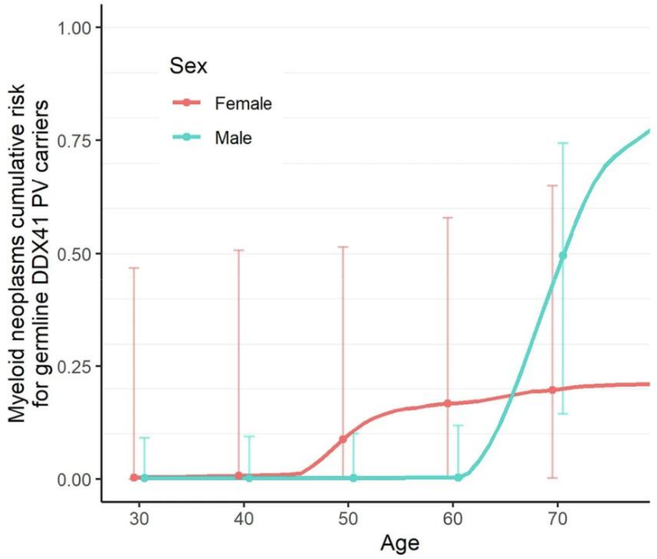


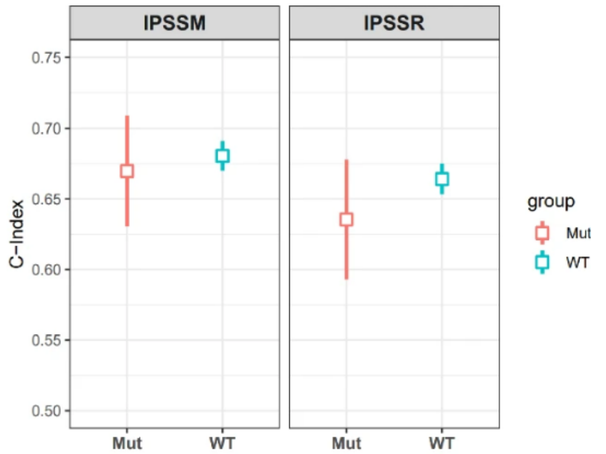




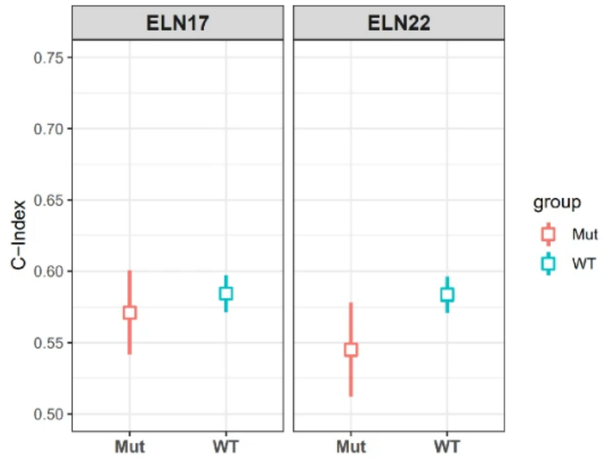
Makishima H et al. Germ line DDX41 mutations define a unique subtype of myeloid neoplasms, Blood 2023
Sébert et al, Blood 2019 and Leukemia 2024
Duployez et al, Blood 2022.

Risk of MDS/AML in *DDX41* carriers and prognostic score





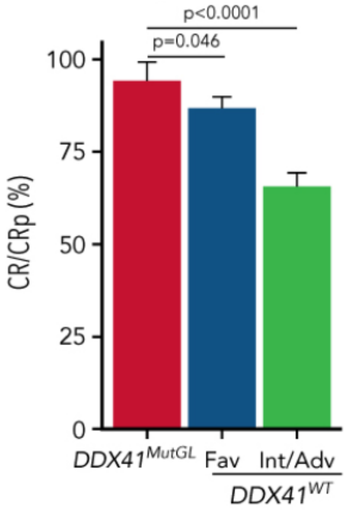
MDS



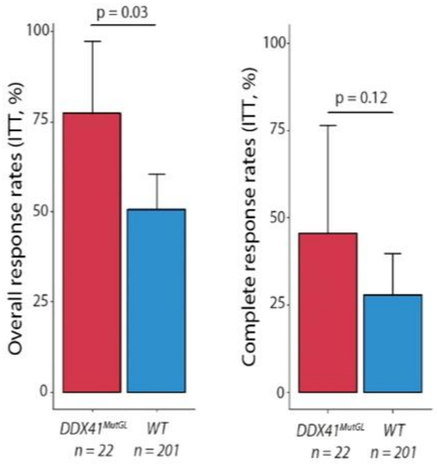
AML

Response to

Chemotherapy



Azacitidine



OS and PFS

Molecular features

Specific germline and somatic *DDX41* mutational configurations

Somatic *DDX41* p.Arg525His (R525H)
Associated with adverse outcomes (across survival endpoints).

Germline truncating *DDX41* variants
Particularly relevant to leukemic evolution.

Combined truncating and somatic R525H variants
Associated with substantially faster leukemic progression.

Phenotypic features

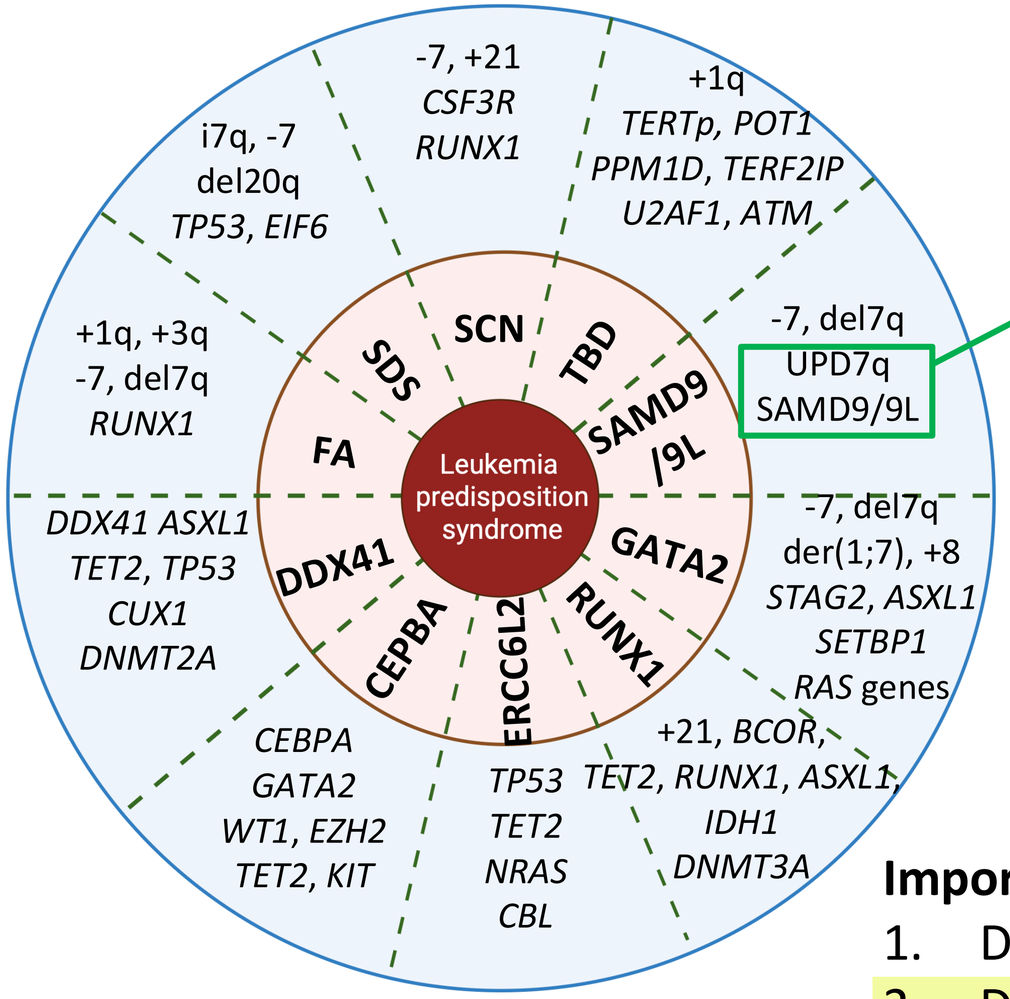
Bone marrow blasts
Percentage of blasts in the bone marrow.

Age
Age at diagnosis (continuous variable).

Sex
Male or female.

Villy et al, EJHG 2025, Gurnari C. et al, Leukemia 2026, Duployez et et al, Blood 2022; Sébert et al, Leukemia 2024.

Clonal trajectories in MDS with germline predisposition



somatic rescue in 64% of SAMD9/9L syndromes

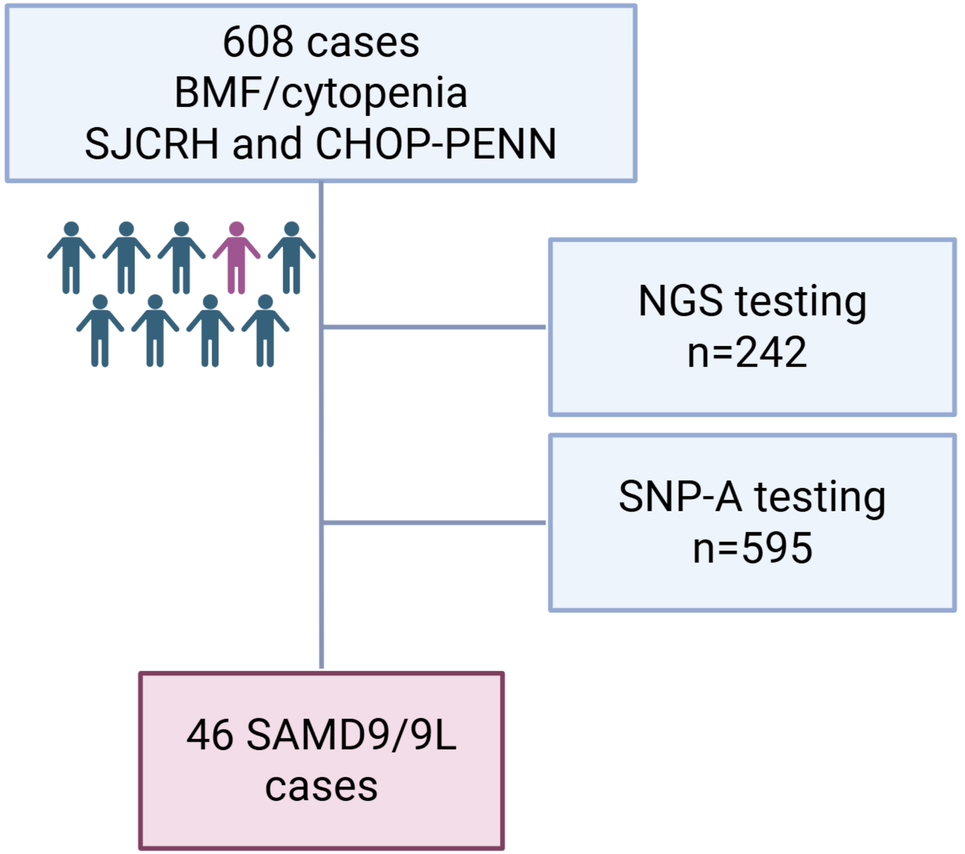
Important for:

1. Diagnosis (red flags)
2. Disease confirmation
3. Disease monitoring (MDS/AML)

The diagnostic value of rescue CH in SAMD9/9L syndromes

Disease-causing germline *SAMD9/9L* variants are primarily missense GoF with no impact on RNA or protein expression levels.
Poor conservation of both genes complicates interpreting the pathogenic effect of these variants.

Based on our internal experience, we assumed that somatic mutations affecting *SAMD9* and *SAMD9L* are specific for SAMD9/9L syndromes and that UPD7q can be either in SAMD9/9L syndromes and Shwachman-Diamond Syndrome.



Attardi E, Babushok D, Wlodarski MW. Manuscript in preparation

St. Jude Children's Research Hospital
BMF/MDS Program
Bone Marrow Failure and Myelodysplastic Syndromes

The diagnostic value of rescue CH in SAMD9/9L syndromes

Somatic SAMD9/SAMD9L

somatic	germline SAMD9/9L	other diagnosis
SAMD9/9L pos	26	0
SAMD9/9L neg	12	204

Sensitivity=[a/(a+c)]×100=68.4%
Specificity=[d/(b+d)]×100=100%
Positive predictive value (PPV)=100%
Negative predictive value (NPV=94.2%

UPD7q, pre- diagnosis of SDS

	germline SAMD9/9L	other diagnosis
UPD7q pos	10	3
UPD7q neg	32	550

Sensitivity=23.8%
Specificity=99.5%
PPV=76.9%
NPV=94.5%

UPD7q, post- diagnosis of SDS

	germline SAMD9/9L	other diagnosis
UPD7q pos	10	0
UPD7q neg	32	535

Sensitivity=23.8%
Specificity=100%
PPV=100%
NPV=94.3%

Monosomy 7

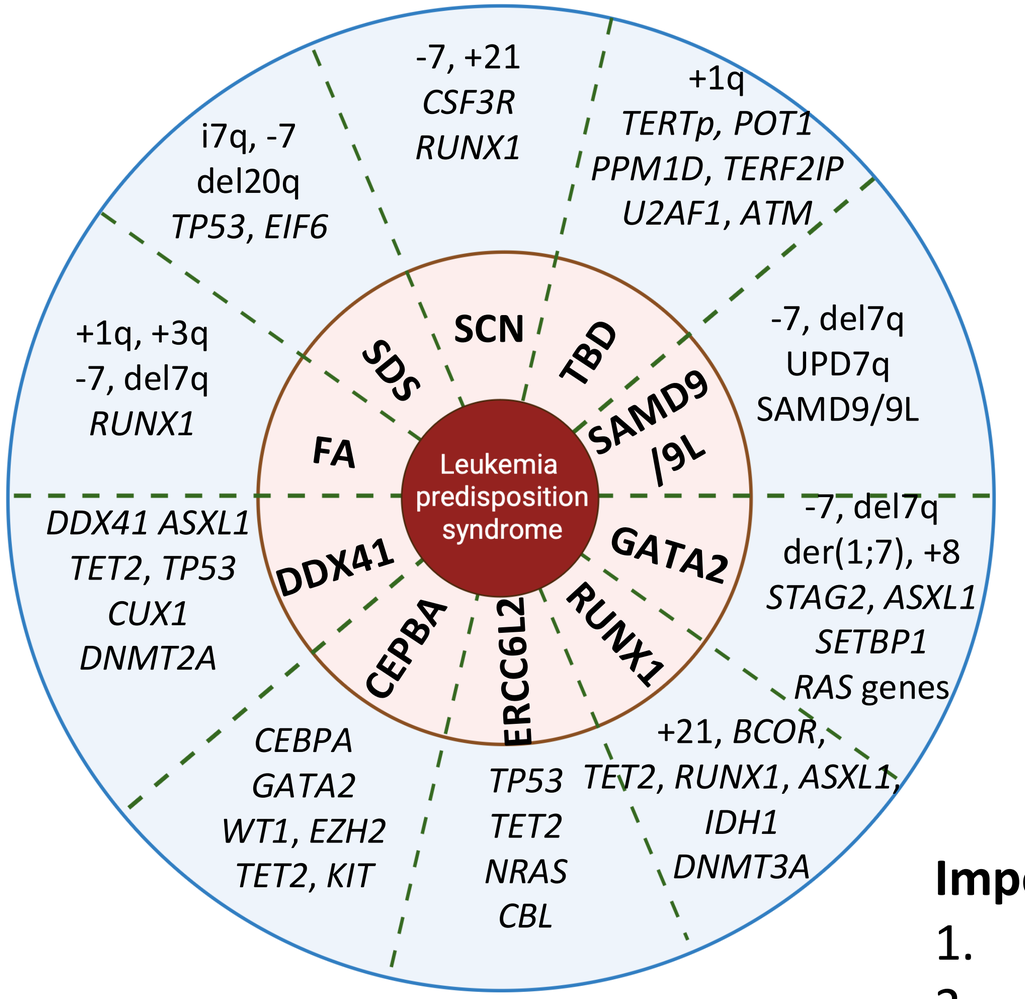
	germline SAMD9/9L	other diagnosis
-7 pos	11	9
-7 neg	31	544

Sensitivity=26.2%
Specificity=98.4%
PPV=55.0%
NPV=94.6%

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Clonal trajectories in MDS with germline predisposition



Important for:

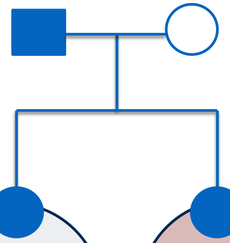
1. Diagnosis (red flags)
2. Disease confirmation
3. Disease monitoring (MDS/AML)

3. Clinical case



2024

- 34-year-old female
- Onset: prolonged neutropenia (and lymphopenia) since 17 yrs of age.
- Blood counts at onset: Hb 13.0 gr/dL, MCV 94, HCT 39%, WBC 2960/uL (**N 860/uL ,** L 800/uL), plts 164000 /uL.
- Personal history: recurrent infections, lymphedema, Hashimoto thyroiditis
- AM/BOM: compatible with MDS-Hypoplastic, normal K



2026 referral

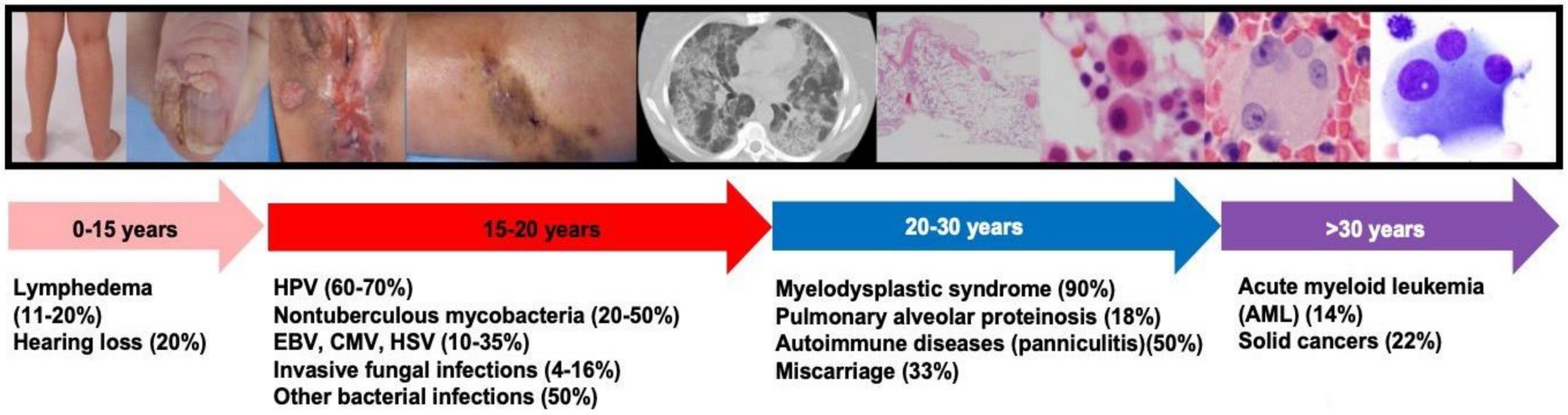
- Blood counts: Hb 13.6 gr/dL, MCV 96.6, WBC 2190/uL (**N 900**, L 1100, M 50), plts 166 000/uL
- Karyotype: normal
- Phenotype: **low absolute B and NK cells**
- NGS extended panel: ***ASXL1*** p.(Gly651Hisfs*3), VAF 4.3%, ***GATA2* p.(Thr354Met) VAF 48.9%,**
- Sanger on CD3+: germline mutation *GATA2* c.1061 C>T p.(Thr354Met)

- Sister, 26 yrs
- Normal bloods count to date
- BM biopsy: **hypocellular**
- Karyotype: normal
- Phenotype: low absolute NK cells
- NGS extended panel**: *GATA2* p.(Thr354Met), VAF 48.3%, *STAG2*** p.(Gly129Argfs*9) VAF 4.1%



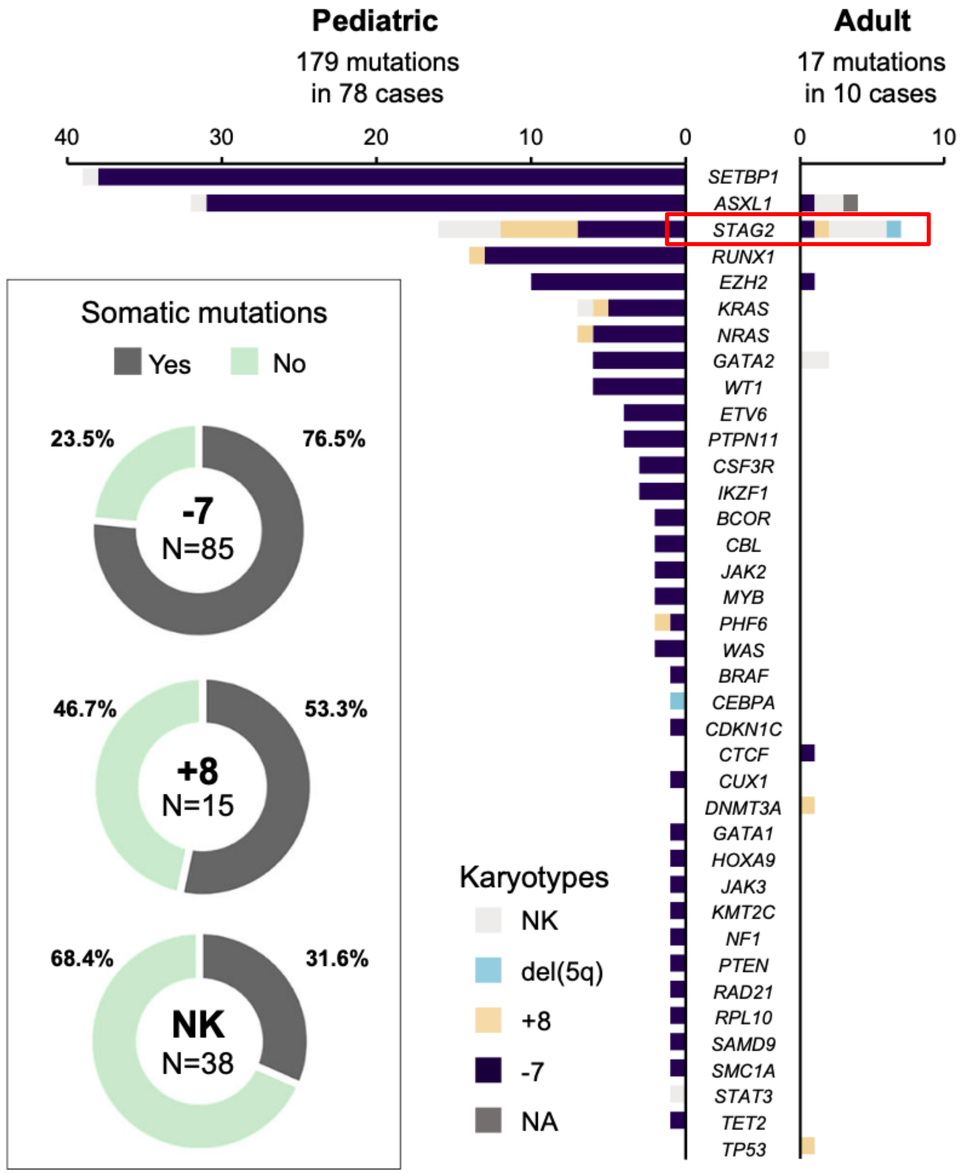
GATA2 deficiency

- Different inheritance patterns, AD or arising *de novo* in 80% of cases → caution considering the familial history
  - 75% of *GATA2* mutation carriers develop MN at an estimated **median age of 20 years**
  - **monosomy 7** in up to 80% of *GATA2*-related MDS patients
- **Immunodeficiency**: particularly reduced or absent dendritic cells, B cells, and NK cells (recurrent infections: especially viral (e.g., HPV, EBV, CMV), fungal, and mycobacterial)
  - Hematologic abnormalities: **neutropenia, lymphopenia, monocytopenia**
  - **HPV-related** disease: persistent HPV infection and extensive warts are characteristic
- Other manifestations: **lymphedema**, hearing abnormalities, and vascular or **autoimmune** complications.



Adapted from B. Terrier

Putative protective role of *STAG2* in GATA2 deficiency



STAG2 mutations are implicated as frequent somatic changes in **clinically stable GATA2** patients and are rarely observed in patients with myeloid malignancy:

- their frequency increases with age and appears **protective against** early development of advanced MDS;
- these clonal events likely represent pre-malignant changes with indeterminate potential that may initially provide a **compensatory mechanism;**
- patients with these mutations are older and have less co-occurring somatic events.

STAG2 mutations may confer selective advantage to GATA2-deficient hematopoietic cells and function more as a rescue rather than transformative events.

Kotmayer L, Attardi E, Wlodarski MW et al. Age-dependent phenotypic and molecular evolution of pediatric MDS arising from GATA2 deficiency. Blood Cancer Journal 2025.

Take-home messages

- The landscape of **germline predisposition to MDS** is increasing overtime. The advent of high-throughput sequencing techniques has enabled rapid genomic profiling that will likely uncover **additional genes**, although establishing the **real pathogenic variants remains challenging** → clinical driven approach

- Missing **germline predisposing variants** has important clinical consequences: such patients are at increased **risk of other organ involvement** and malignancy, and these patients require **disease-specific monitoring**. Understanding the interplay between germline predisposition and **clonal selection** can support more personalized strategies for surveillance

GIMEMA AML2120

AIMS OF THE TRIAL

PRIMARY OBJECTIVE

To evaluate the incidence of leukemia predisposition syndromes in patients with MDS or AML with almost one familial or personal sign of germline predisposing condition.

UNDERSTANDING GERMLINE PREDISPOSITION TO AML/MDS

1. **Describing the clinical, biological and hematological characteristics** of patients with germline predisposition to AML and MDS to provide a scalable model for optimizing the detection of germline predisposing variants.
2. **Distribution of germline variants** determining leukemia predisposition syndromes.
3. **Recurrence of somatic clonal events** in patients with germline predisposition to AML/MDS and definition of specific somatic pattern of malignant clones determining leukemia transformation.
4. **Incidence of transplantation procedure** in patients with familial AML/MDS.
5. **Overall Survival (OS) and Progression Free Survival (PFS).**
6. To compare the survival risk in patients with germline predisposition to AML/MDS with the risk in the italian general population.

Generating evidence to improve identification, risk stratification and outcomes of patients with germline predisposition to AML/MDS.

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