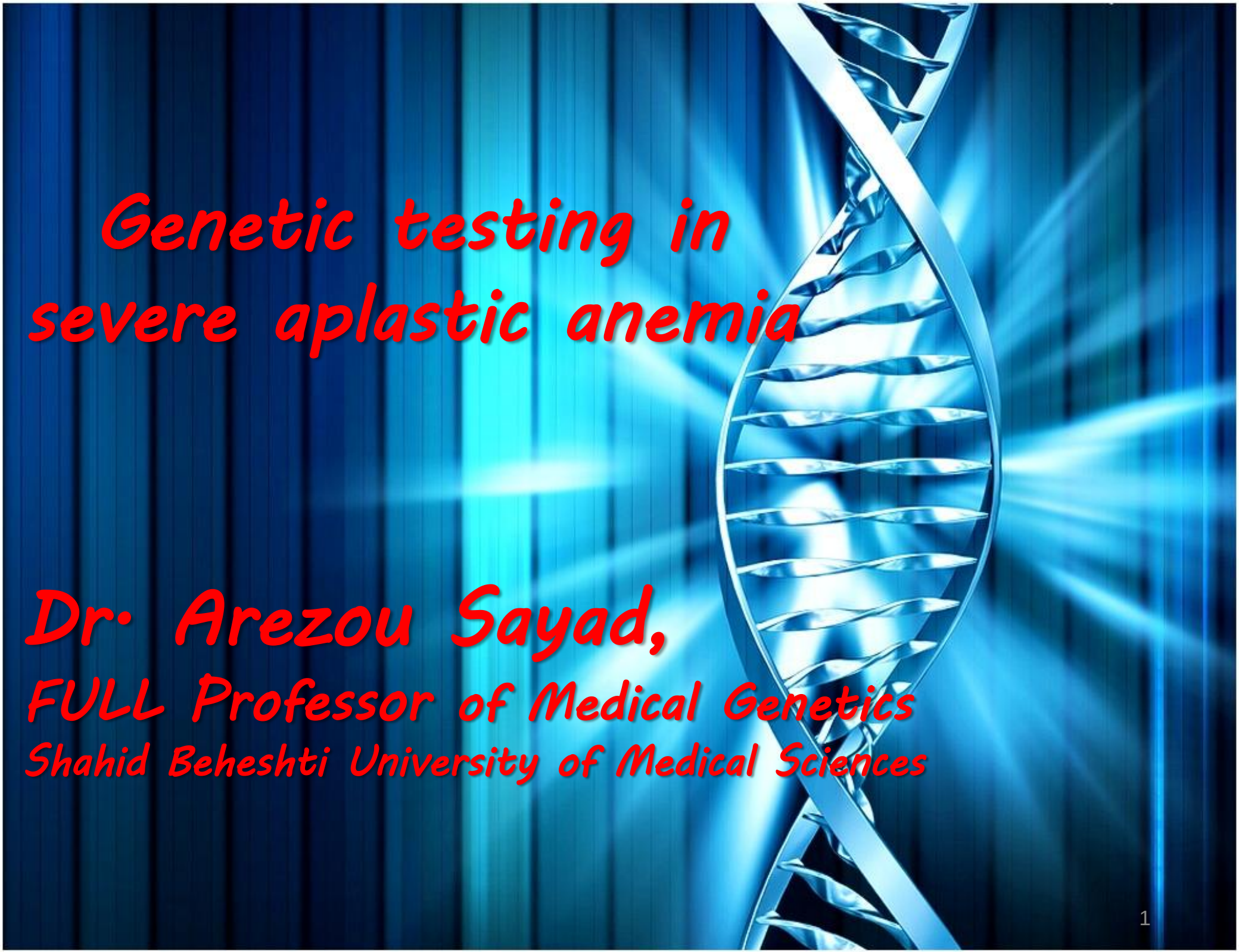

Genetic testing in severe aplastic anemia

Dr. Arezou Sayad,
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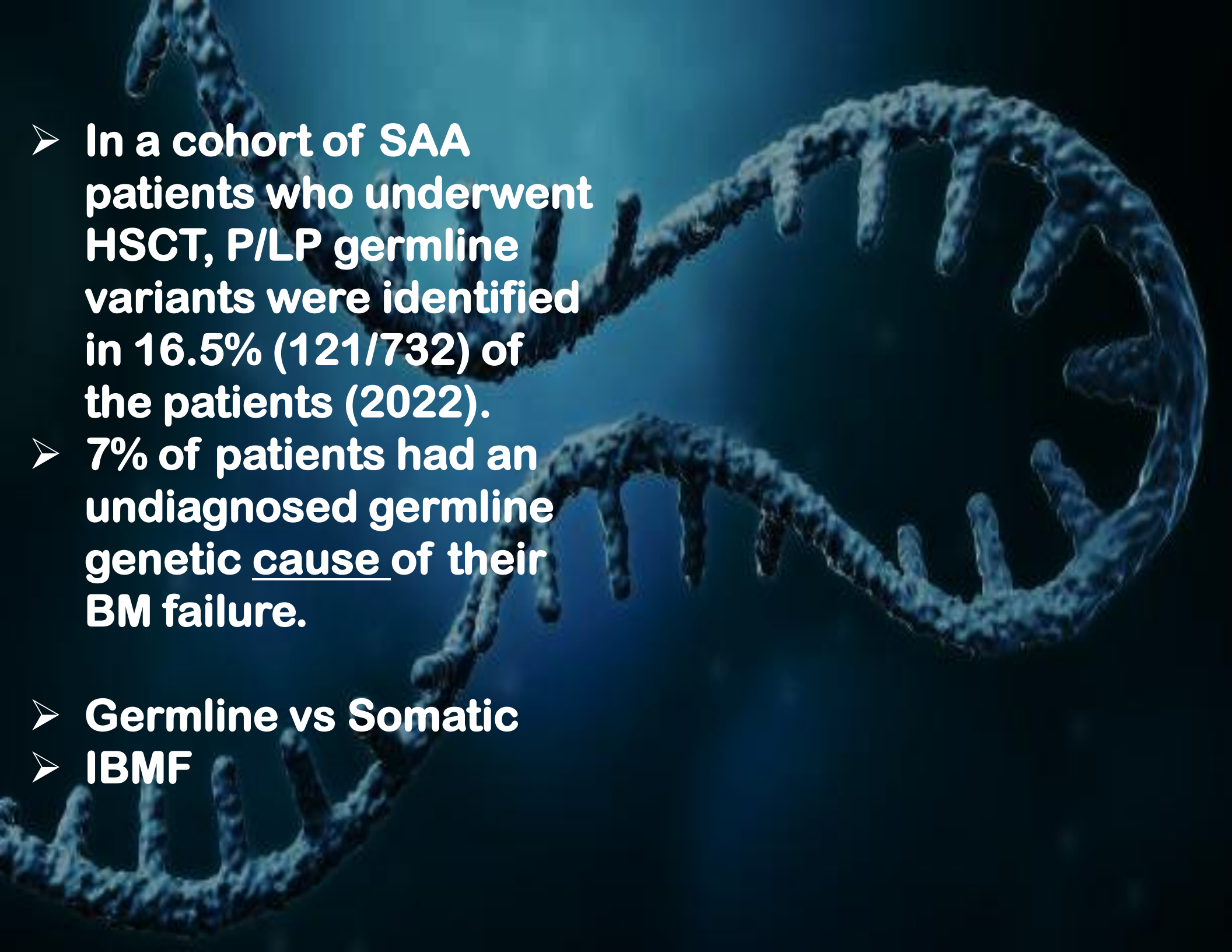


- In a cohort of SAA patients who underwent HSCT, P/LP germline variants were identified in 16.5% (121/732) of the patients (2022).
- 7% of patients had an undiagnosed germline genetic cause of their BM failure.

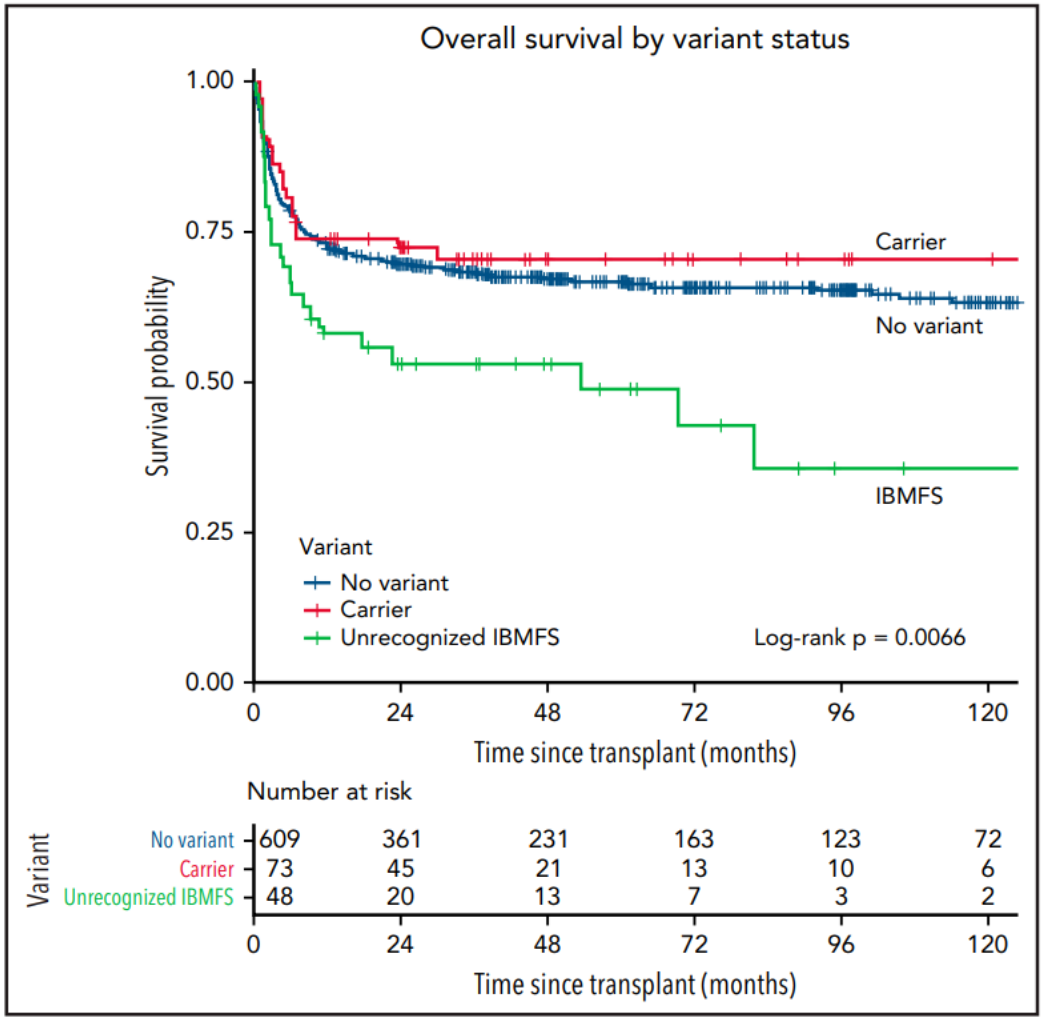
- Germline vs Somatic
- IBMF

Associated genes with IBMF:

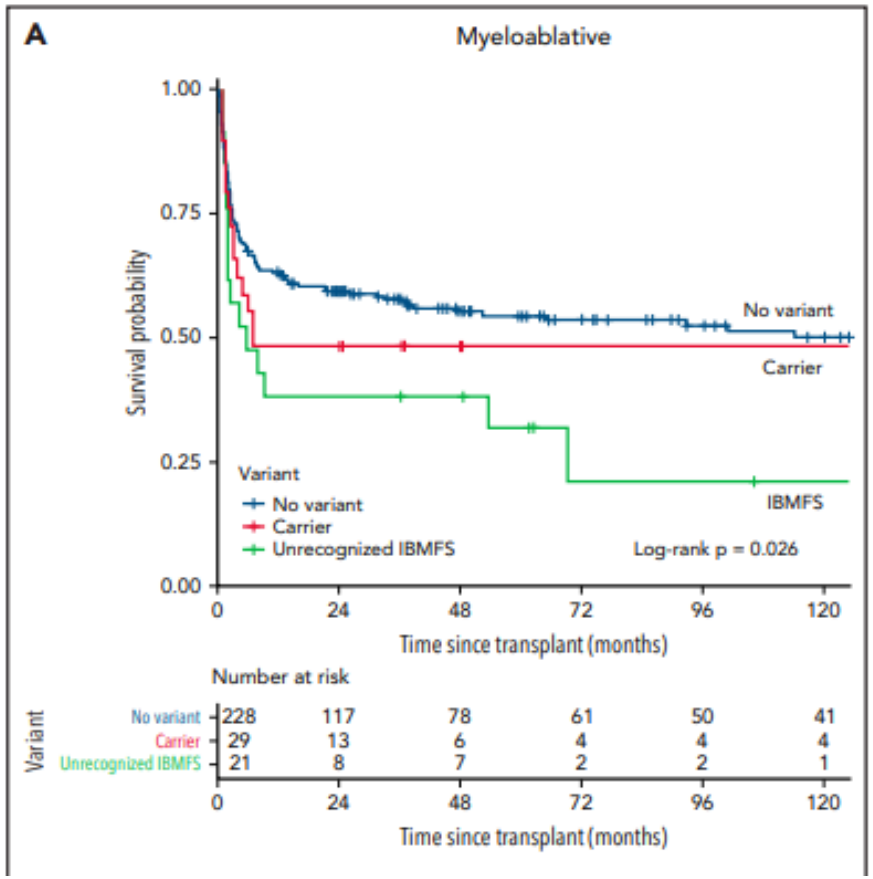
1. DNA damage response (FA)
2. Telomere related Disorders
   (Dyskeratosis congenita)
3. ribosome biology
   (Diamond Blackfan,Shwachman Diamond)
4. Hematopoiesis related genes

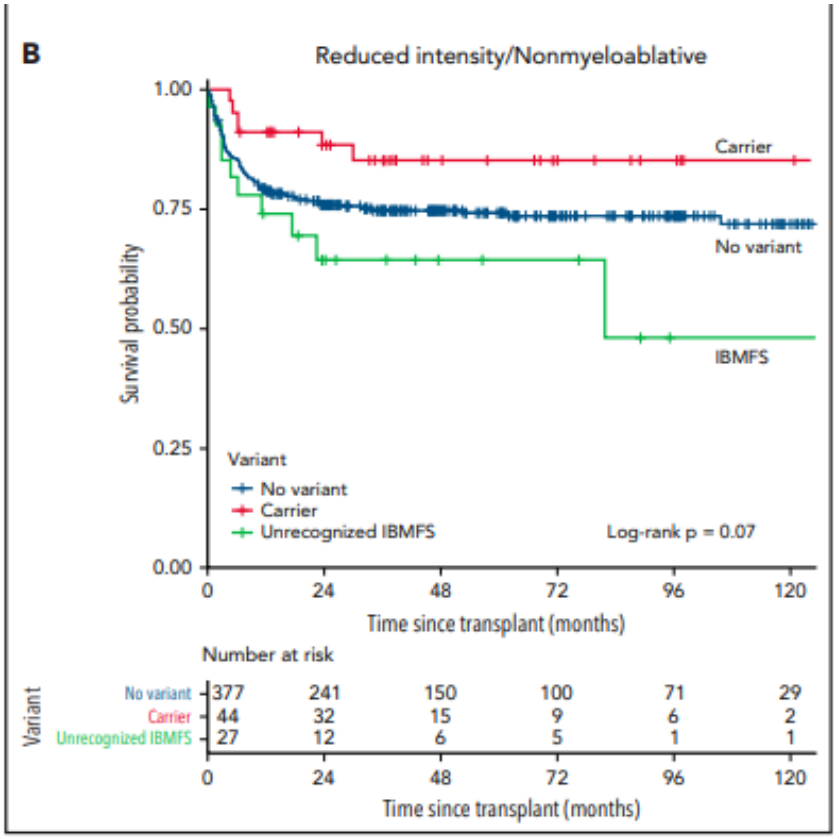
- Chromosome breakage testing does not identify all **DNA damage** response disorders(ERCC6L2 or LIG4).

Con...


Overall survival by variant status
1.00
0.75
0.50
0.25
0.00
Survival probability
Carrier
No variant
IBMFS
Variant
No variant
Carrier
Unrecognized IBMFS
Log-rank p = 0.0066
0
24
48
72
96
120
Time since transplant (months)
Number at risk
Variant
No variant 609 361 231 163 123 72
Carrier 73 45 21 13 10 6
Unrecognized IBMFS 48 20 13 7 3 2
0
24
48
72
96
120
Time since transplant (months)

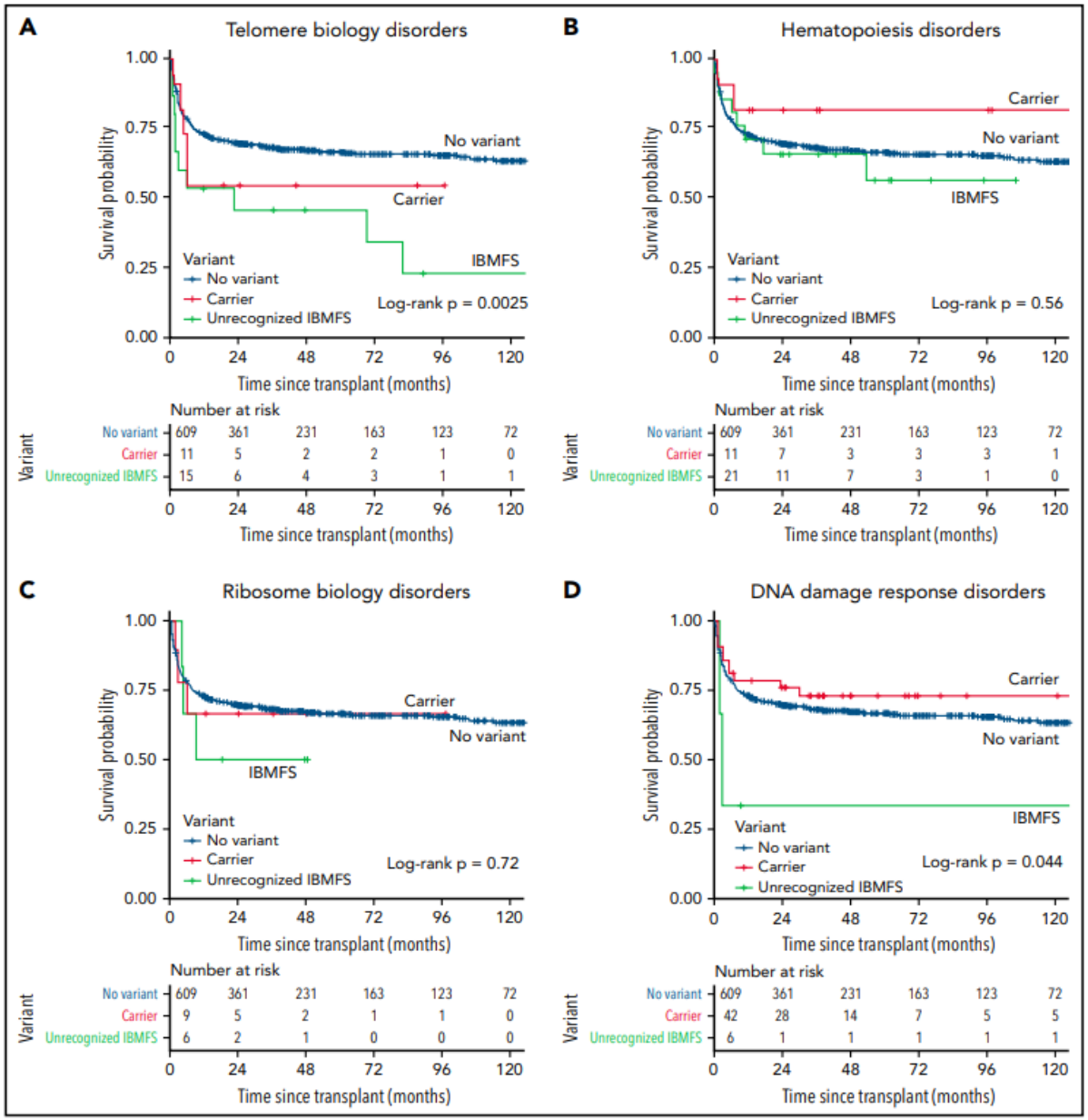
Con...

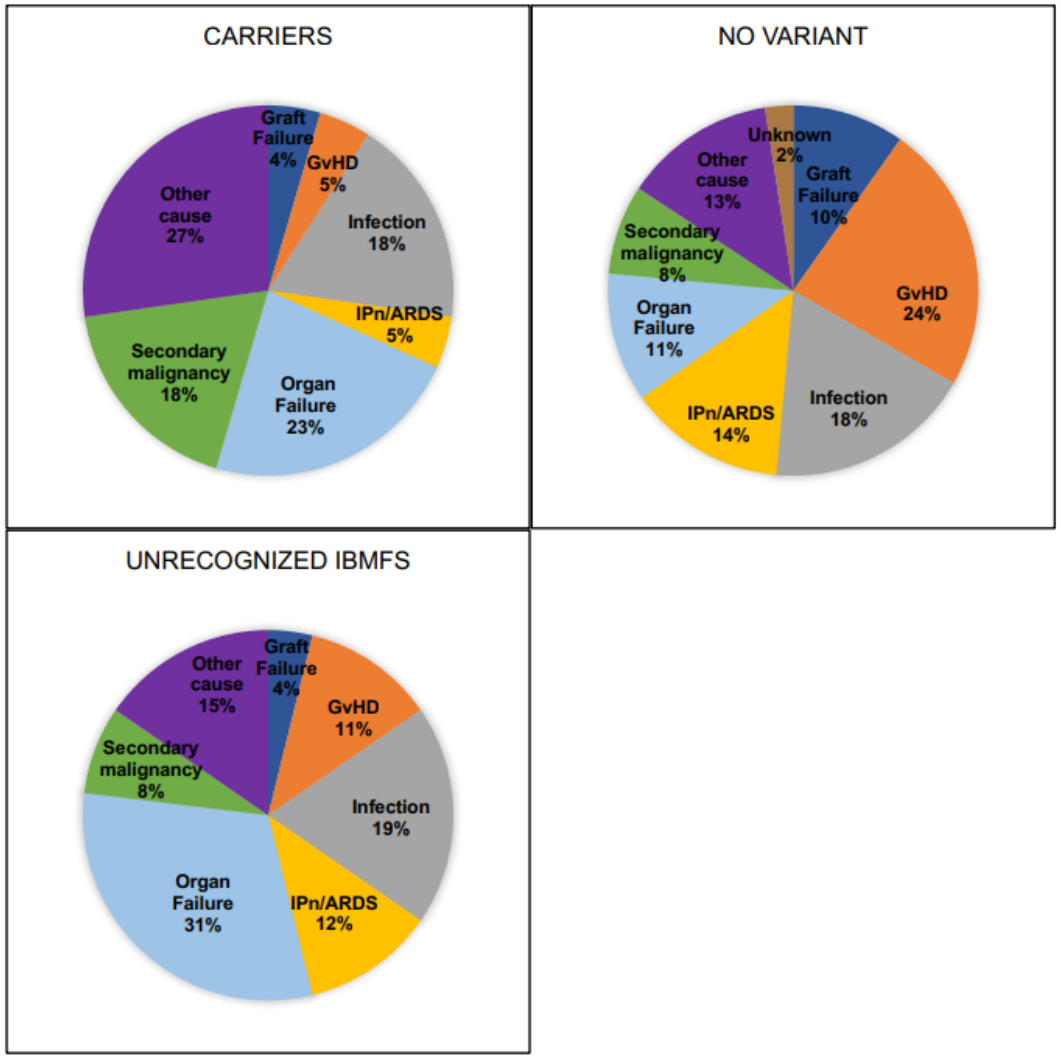




- while nonmyeloablative regimens are now standard for SAA, 40% of unrecognized IBMFS patients received myeloablative regimens.

Con...


A
Telomere biology disorders
Survival probability
1.00
0.75
0.50
0.25
0.00
No variant
Carrier
IBMFS
Variant
No variant
Carrier
Unrecognized IBMFS
Log-rank p = 0.0025
0 24 48 72 96 120
Time since transplant (months)
Number at risk
Variant
No variant 609 361 231 163 123 72
Carrier 11 5 2 2 1 0
Unrecognized IBMFS 15 6 4 3 1 1
0 24 48 72 96 120
Time since transplant (months)
B
Hematopoiesis disorders
Survival probability
1.00
0.75
0.50
0.25
0.00
Carrier
No variant
IBMFS
Variant
No variant
Carrier
Unrecognized IBMFS
Log-rank p = 0.56
0 24 48 72 96 120
Time since transplant (months)
Number at risk
Variant
No variant 609 361 231 163 123 72
Carrier 11 7 3 3 3 1
Unrecognized IBMFS 21 11 7 3 1 0
0 24 48 72 96 120
Time since transplant (months)
C
Ribosome biology disorders
Survival probability
1.00
0.75
0.50
0.25
0.00
Carrier
No variant
IBMFS
Variant
No variant
Carrier
Unrecognized IBMFS
Log-rank p = 0.72
0 24 48 72 96 120
Time since transplant (months)
Number at risk
Variant
No variant 609 361 231 163 123 72
Carrier 9 5 2 1 1 0
Unrecognized IBMFS 6 2 1 0 0 0
0 24 48 72 96 120
Time since transplant (months)
D
DNA damage response disorders
Survival probability
1.00
0.75
0.50
0.25
0.00
Carrier
No variant
IBMFS
Variant
No variant
Carrier
Unrecognized IBMFS
Log-rank p = 0.044
0 24 48 72 96 120
Time since transplant (months)
Number at risk
Variant
No variant 609 361 231 163 123 72
Carrier 42 28 14 7 5 5
Unrecognized IBMFS 6 1 1 1 1 1
0 24 48 72 96 120
Time since transplant (months)



Pie charts comparing the causes of death in carriers versus unrecognized IBMFS patients versus patients with no variants.

Test	At diagnosis	Purpose	Notes	References[1]
Telomere length measurement of peripheral blood lymphocytes by Flow-FISH	X (age ≤40 y or those proceeding to BMT)	Determine lymphocyte telomere length by Flow-FISH; if less than first percentile, patient may have an STS and be at risk for increased transplant-related toxicity with standard preparative regimens, and an STS-specific regimen should be considered.	Lymphocyte telomere lengths less than first percentile are highly sensitive and specific for an STS diagnosis in young patients with AA. **Caveats:** Other IBMFDs can have lengths less than or equal to the first percentile; individuals with pathogenic telomere gene mutations can have lengths in the normal range, usually between the first and tenth percentiles; and short telomeres can be seen in acquired AA with reduced stem cell reserve.	66-68,86
Chromosome breakage analysis on peripheral blood	X (age ≤40 y or those proceeding to BMT)	Evaluate for FA; if test is positive and consistent with a diagnosis of FA, patient is at increased risk for transplant-related toxicity, and an FA-specific regimen should be used.	If results are normal but clinical suspicion remains high, this test can be performed on cultured skin fibroblasts to rule out a false negative in the peripheral blood.	87
Conventional karyotyping	X		Most often AA patients have normal cytogenetics but there are some cytogenetic abnormalities seen in AA that are not considered adverse or indicative of MDS (in the absence of dysplasia). These include del13q, trisomy 8, loss of heterozygosity of short arm of chromosome 6, among others. If <20 metaphases are obtained, perform an MDS FISH panel; a microarray can be considered as an alternative in these cases. Monosomy 7, especially in young patients, increases suspicion for an IBMFD.	40,88

Con...

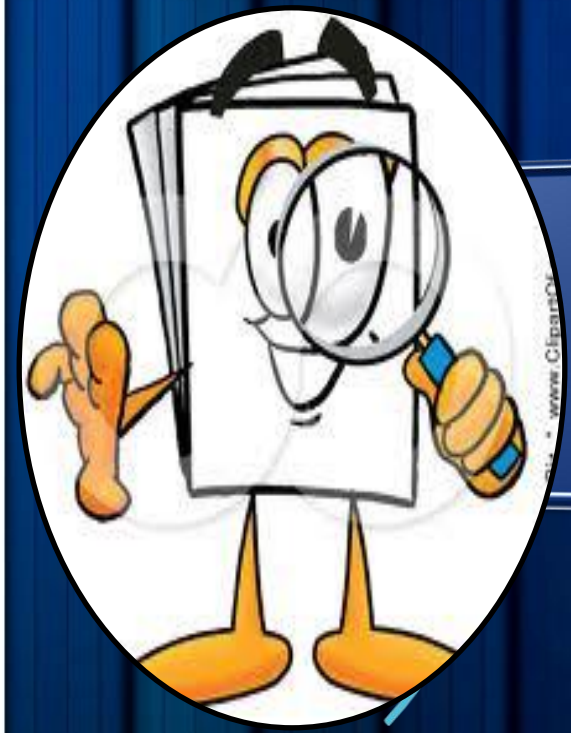
Test	At diagnosis	Purpose	Notes	References[15,19]
Myeloid malignancy gene sequencing from peripheral blood or bone marrow	Strongly consider if any concern for possible hypoplastic MDS	Evaluate for mutations in genes recurrently mutated in AA (eg, *PIGA*, *BCOR*, *BCORL1*) and/or MDS (eg, epigenetic mutations, *TP53*).	Identification of mutations should not necessarily be used as a discriminating tool between AA and hypoplastic MDS, because most MDS-associated mutations (including *BCOR*, *BCORL1*, and epigenetic mutations, such as *DNMT3A*, *TET2*, *ASXL1*, and others) are seen in AA, MDS, and aging-related clonal hematopoiesis and have poor discriminating power for AA or MDS in this context. Acquired mutation panel may identify a subset of AA likely to progress to MDS/AML. A portion of the genes on these acquired panels overlap with inherited marrow failure gene panels but they should not be considered adequate testing for IBMFD as a stand-alone test.	52,56,89
Inherited BMF gene panel sequencing	Patients aged ≤40 y or if clinical picture or screening tests warrant	Evaluate for multiple IBMFDs at once. Overlapping phenotypes and lack of physical features and family history in a substantial subset of those with IBMFD makes universal testing of young patients warranted. Tissue source for this testing should ideally be cultured skin fibroblasts (see text for discussion).	Yield in adult patients with AA aged 18-40 y is 5-15%. Yield increases with the presence of phenotypic features or family history or in cases where hypoplastic MDS is a consideration (eg, monosomy 7).	13,14

Donor Selection

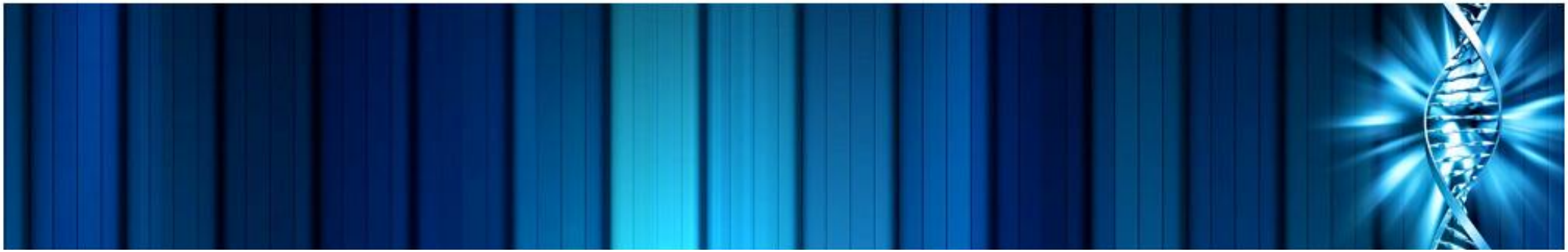
Ziba K
4-6 weeks

Genetic testing in severe aplastic anemia is required for optimal hematopoietic cell transplant outcomes

THANKS



- Patients with SAA because of IBMFS do not respond to IST, further illustrating the importance of early genetic diagnosis to guide therapeutic decision.
- Variant Allele frequency (VAF)
- germline predisposition for bone marrow failure, **DNA repair**, and **telomere** biology disorders occurred at ages of **<40** years, whereas **checkpoint disorders** or germline variants in **DDX41** occurred at a **later age**.



ID	Age Group	HCT Time Frame	Gene Group	Gene	Inheritance	Variant Type(s)	Allele 1 Variant	Transcript	HGVS c.	HGVS p.
UIBMFS-1	10-20	2011-2015	DNA Damage Response Disorder	*FANCA*	Compound Heterozyous	SNV/SNV	chr16:89858442 delCCAA	NM_000135.4	c.1115_1118delTTGG	V372Afs*42 (p.Val372AlafsTer42)
UIBMFS-2	10-20	2011-2015	DNA Damage Response Disorder	*FANCA*	Compound Heterozyous	SNV/CNV	chr16:89828357 C⇒T	NM_000135.4	c.2852G>A	R951Q (p.Arg951Gln)
UIBMFS-3	10-20	2001-2005	DNA Damage Response Disorder	*ERCC6L2*	Compound Heterozyous	SNV/SNV	chr9:98678598 delAG	NM_001010895.4	c.1045_1046delGA	E349Tfs*33 (p.Glu349ThrfsTer33)
UIBMFS-4	10-20	1996-1999	DNA Damage Response Disorder	*ERCC6L2*	Compound Heterozyous	SNV/SNV	chr9:98678608 delAGCC	NM_001010895.4	c.1051_1054delGCCA	A351Lfs*41 (p.Ala351LeufsTer41)
UIBMFS-5	20-30	2006-2010	DNA Damage Response Disorder	*ERCC6L2*	Compound Heterozyous	SNV/CNV	chr9:98678608 delAGCC	NM_001010895.4	c.1051_1054delGCCA	A351Lfs*41 (p.Ala351LeufsTer41)
UIBMFS-6	10-20	2006-2010	DNA Damage Response Disorder	*LIG4*	Compound Heterozyous	SNV/SNV	chr13:108862104 delGA	NM_206937.2	c.1512_1513delTC	R505Cfs*12 (p.Arg505CysfsTer12)
UIBMFS-7	0-10	2001-2005	Ribosome Biology Disorder	*DNAJC21*	Homozygous	SNV	chr5:34935912_3 insA	NM_001012339.3	c.290dupA	Y97* (p.Tyr97Ter)
UIBMFS-8	0-10	2006-2010	Ribosome Biology Disorder	*DNAJC21*	Homozygous	SNV	chr5:34941289 G⇒A	NM_001012339.3	c.983+1G>A	
UIBMFS-9	10-20	2006-2010	Ribosome Biology Disorder	*SBDS*	Compound Heterozyous	SNV/CNV	chr7:66459197 A⇒G	NM_016038.4	c.258+2T>C	
UIBMFS-10	10-20	2011-2015	Ribosome Biology Disorder	*RPS24*	Heterozygous	SNV	chr10:79814467 T⇒A	NM_001142285.2	c.569T>A	L190* (p.Leu190Ter)
UIBMFS-11	0-10	1996-2000	Ribosome Biology Disorder	*RPS19*	Heterozygous	SNV	chr19:42364847 G⇒A	NM_001022.4	c.3G>A	M1I (p.Met1Ile)
UIBMFS-12	20-30	2011-2015	Ribosome Biology Disorder	*RPS19*	Heterozygous	SNV	chr19:42373818 G⇒A	NM_001022.4	c.406G>A	G136R (p.Gly136Arg)
UIBMFS-13	0-10	1996-2000	Telomere Biology Disorder	*DKC1*	Hemizygous	SNV	chrX:153993734 delGAA	NM_001363.5	c.103_105delGAA	E35del (p.Glu35del)
UIBMFS-14	30-40	1996-2000	Telomere Biology Disorder	*WRAP53*	Compound Heterozyous	SNV/SNV	chr17:7606139 C⇒T	NM_001143990.1	c.1243C>T	Q415* (p.Gln415Ter)
UIBMFS-15	10-20	2006-2010	Telomere Biology Disorder	*TERT*	Compound Heterozyous	SNV/SNV	chr5:1255486 C⇒A	NM_198253.3	c.3073G>T	V1025F (p.Val1025Phe)
UIBMFS-16	0-10	2006-2010	Telomere Biology Disorder	*TERT*	Compound Heterozyous	SNV/SNV	chr5:1266605 G⇒C	NM_198253.3	c.2628C>G	H876Q (p.His876Gln)
UIBMFS-17	10-20	1991-1995	Telomere Biology Disorder	*TERT*	Heterozygous	SNV	chr5:1278895 G⇒A	NM_198253.3	c.2147C>T	A716V (p.Ala716Val)
UIBMFS-18	20-30	2011-2015	Telomere Biology Disorder	*TERT*	Heterozygous	SNV	chr5:1294493 C⇒T	NM_198253.3	c.508G>A	V170M (p.Val170Met)
UIBMFS-19	0-10	2011-2015	Telomere Biology Disorder	*RTEL1*	Heterozygous	SNV	chr20:62323122 delCTGT	NM_032957.5	c.2659_2662delTCTG	S887Rfs*40 (p.Ser887ArgfsTer40)
UIBMFS-20	0-10	2006-2010	Telomere Biology Disorder	*TINF2*	Heterozygous	SNV	chr14:24709841 C⇒T	NM_001099274.3	c.845G>A	R282H (p.Arg282His)
UIBMFS-21	10-20	1991-1995	Telomere Biology Disorder	*TINF2*	Heterozygous	SNV	chr14:24709842 G⇒A	NM_001099274.3	c.844C>T	R282C (p.Arg282Cys)
UIBMFS-22	0-10	1996-2000	Telomere Biology Disorder	*TINF2*	Heterozygous	SNV	chr14:24709842 G⇒A	NM_001099274.3	c.844C>T	R282C (p.Arg282Cys)
UIBMFS-23	0-10	2001-2005	Telomere Biology Disorder	*TINF2*	Heterozygous	SNV	chr14:24709818_9 insG	NM_001099274.3	c.867dupC	F290Lfs*2 (p.Phe290LeufsTer2)
UIBMFS-24	20-30	2006-2010	Telomere Biology Disorder	*TINF2*	Heterozygous	SNV	chr14:24709828_9 insA	NM_001099274.3	c.857dupT	M286Ifs*6 (p.Met286IlefsTer6)
UIBMFS-25	0-10	1991-1995	Telomere Biology Disorder	*TINF2*	Heterozygous	SNV	chr14:24709824 A⇒G	NM_001099274.3	c.862T>C	F288L (p.Phe288Leu)
UIBMFS-26	30-40	2001-2005	Telomere Biology Disorder	*TERC*	Heterozygous	SNV	chr3:169482530 C⇒T	NR_001566.1	r.319G>A	
UIBMFS-27	30-40	2006-2010	Telomere Biology Disorder	*POT1*	Compound Heterozyous	CNV/CNV	chr7:124481007-124481252	NM_001042594.1	Deletion of exon 13 (246 bp)	
UIBMFS-28	0-10	2006-2010	Hematopoiesis Disorder	*ADA2*	Homozygous	SNV	chr22:17687997 C⇒T	NM_001282225.2	c.506G>A	R169Q (p.Arg169Gln)
UIBMFS-29	0-10	1996-2000	Hematopoiesis Disorder	*MPL*	Compound Heterozyous	SNV/SNV	chr1:43804235 delCT	NM_005373.3	c.235_236delCT	L79Efs*84 (p.Leu79GlufsTer84)
				THPO	Heterozygous	SNV	chr3:184093356 G⇒A	NM_000460.4	c.175C>T	P59S (p.Pro59Ser)
UIBMFS-30	0-10	2006-2010	Hematopoiesis Disorder	*MPL*	Compound Heterozyous	SNV/SNV	chr1:43804305 G⇒C	NM_005373.3	c.305G>C	R102P (p.Arg102Pro)
UIBMFS-31	0-10	2006-2010		*MPL*	Compound Heterozyous	SNV/SNV	chr1:43804305 G⇒C	NM_005373.3	c.305G>C	R102P (p.Arg102Pro)
UIBMFS-32	10-20	1996-2000	Hematopoiesis Disorder	*MPL*	Compound Heterozyous	SNV/SNV	chr1:43804305 G⇒C	NM_005373.3	c.305G>C	R102P (p.Arg102Pro)
UIBMFS-33	30-40	2001-2005	Hematopoiesis Disorder	*MPL*	Compound Heterozyous	SNV/SNV	chr1:43805106 C⇒T	NM_005373.3	c.556C>T	Q186* (p.Gln186Ter)
UIBMFS-34	30-40	2006-2010	Hematopoiesis Disorder	*GATA2*	Heterozygous	SNV	chr3:128200155_6 insCTAG	NM_032638.5	c.1149_1150insCTAG	R384Lfs*153 (p.Arg384LeufsTer153)
UIBMFS-35	10-20	2001-2005	Hematopoiesis Disorder	*MECOM*	Heterozygous	SNV	chr3:168845687 delTC	NM_004991.4	c.774_775delGA	E258Dfs*8 (p.Glu258AspfsTer8)
UIBMFS-36	20-30	1996-2000	Hematopoiesis Disorder	*MECOM*	Heterozygous	SNV	chr3:168861485 C⇒T	NM_004991.4	c.510+1G>A	
UIBMFS-37	10-20	2011-2015	Hematopoiesis Disorder	*HOXA11*	Heterozygous	SNV	chr7:27224508 C⇒A	NM_005523.6	c.256G>T	E86* (p.Glu86Ter)
UIBMFS-38	10-20	2001-2005	Hematopoiesis Disorder	*ETV6*	Heterozygous	SNV	chr12:12022535 C⇒T	NM_001987.5	c.641C>T	P214L (p.Pro214Leu)
UIBMFS-39	20-30	2006-2010	Hematopoiesis Disorder	*RUNX1*	Heterozygous	SNV	chr21:36171708_9 insG	NM_001754.4	c.856dupC	Q286Pfs*314 (p.Gln286ProfsTer314)
UIBMFS-40	20-30	1996-2000	Hematopoiesis Disorder	*RUNX1*	Heterozygous	SNV	chr21:36252945 delGT…TG (13)	NM_001754.4	c.405_417del	N136Tfs*5 (p.Asn136ThrfsTer5)
UIBMFS-41	30-40	2006-2010	Hematopoiesis Disorder	*RUNX1*	Heterozygous	SNV	chr21:36259174 delACinsAT	NM_001754.4	c.316_317delTGinsAT	W106M (p.Trp106Met)
UIBMFS-42	10-20	2001-2005	Hematopoiesis Disorder	*RUNX1*	Heterozygous	SNV	chr21:36259223 C⇒T	NM_001754.4	c.268G>A	V90M (p.Val90Met)
UIBMFS-43	0-10	2011-2015	Hematopoiesis Disorder	*RUNX1*	Heterozygous	SNV	chr21:36262002 delC	NM_001754.4	c.34delG	E12Kfs*24 (p.Glu12LysfsTer24)
UIBMFS-44	0-10	2011-2015	Hematopoiesis Disorder	*ANKRD26*	Heterozygous	CNV	chr10:27352897-27353030	NM_014915.2	Deletion of exon 12 (134 bp)	
UIBMFS-45	>60	2011-2015	Hematopoiesis Disorder	*ANKRD26*	Heterozygous	CNV	chr10:27322134-27322326	NM_014915.2	Deletion of exon 25 (193 bp)	
UIBMFS-46	50-60	2011-2015	Hematopoiesis Disorder	*ANKRD26*	Heterozygous	CNV	chr10:27355396-27355497	NM_014915.2	Deletion of exon 11 (102 bp)	
UIBMFS-47	10-20	2011-2015	Hematopoiesis Disorder	*MECOM*	Heterozygous	CNV	chr3:168845612-168849339	NM_005241.3	Deletion of exons 3 and 4 (3728 bp)	