Germline predisposition in hematologic malignancies; A new strategy era

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HISTORY

- In 1969, Li and Fraumeni…
- Twenty-one years later, the germ line transmission of a mutant TP53 gene...
- The landmark paper of Li and Fraumeni :

"**cancer predisposition revolution**"

Why testing for *germline predisposition* in HMs?

It can guide therapeutic decisions, appropriate genetic counseling, familial screening, and surveillance

- Somatic mutation
- At least 7% of all **MDS** taken to related allogeneic HSCT carried **P/LP** variants (2022), and 19% of all MDS patients have germline mutation (2021).
- **MDS** patients showed that germline predisposition occurred in patients of any age, even in those aged>70 years.
- 1120 patients with **pediatric cancer** showed that 8.5% had germline mutations in cancer-predisposing genes and only 40% had a family history of cancer.
- In a cohort of SAA patients who underwent HSCT, P/LP germline variants were identified in 16.5% (121/732) of the patients (2022).

Germline predisposition

1. therapeutic modifications, eg. FA **reduced-intensity** conditioning is required as inherent **hypersensitivity** to genotoxic

2. GATA2 deficiency patients: various **infectious** complications during therapy

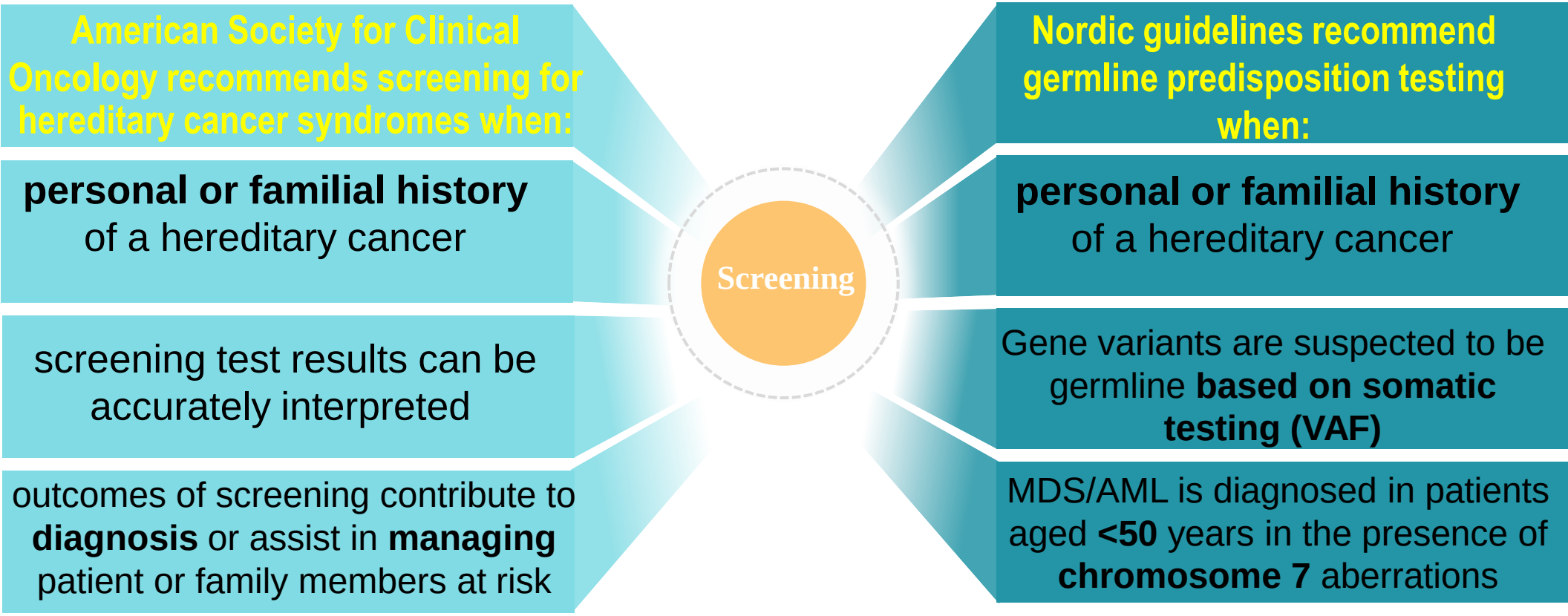
3. **Donor-derived malignancies** have been reported in myeloid neoplasms with germline predisposition for **CEBPA, DDX41** , and **GATA2**

4. challenges in Stem cell donors carrying pathogenic germline variants:
stem cell **mobilization** or delayed **engraftment failure**

2022 European LeukemiaNet :

Germline predisposition should be considered in patients with any HMs, irrespective of age

Universal screening of individuals for germline predisposition of myeloid neoplasms is **not currently the standard of care**, but



Which Sources?

1. **blood, bone marrow, saliva, buccal swabs**, all contain hematopoietic cells and contaminated with malignant cells

2. **hair bulbs** and **Nail** (Low con. DNA)

3. cultured **skin fibroblasts** from a skin punch biopsy

	Pros	Cons	Reference(s)
Cultured skin fibroblasts	Confirmed germline	4–6 weeks turnaround time	DeRoin et al. 2022
	High yield for downstream analysis	Procedure needs training.	
	No donor cell contamination for patients who had HCT	~5% culture failure	
Primary skin fibroblasts (washed extensively)	No culture time	Possibility of blood contamination	NA
Hair bulbs	Confirmed germline	Procedure is painful for patients.	Trottier & Godley 2021
	No culture time	Patients treated with chemotherapy may have alopecia.	
		Low DNA yield	
Peripheral blood	DNA can be extracted directly from the sample.	Not true germline	DeRoin et al. 2022, Trottier & Godley 2021
	High DNA yield	Somatic reversion and CH can give inconclusive results.	
	Sample collection is done regularly and can be easily obtained.		
Bone marrow aspirate	Patients undergo bone marrow biopsy and sample can be easily obtained.	Not true germline	Godley 2023
		Contains hematopoietic cells	
		>95% donor cell contamination for patients who had HCT	
Bone marrow–derived MSCs	Nonhematopoietic; confirmed germline	Requires special culture conditions for cells to grow	Mastrolia et al. 2019
	Adherent to plastic, enabling MSC culture independently of isolating hematopoietic cells for cytogenetic analysis	Growth of MSC cultures varies among patients.	
		Primary culture failure rate 28%	
		Multilineage differentiation can cause loss of gene expression.	
		Cell senescence occurs at passage 5, which limits DNA yield.	
Saliva	DNA can be extracted directly from the sample.	Somatic mutations and CH present in the sample compromise the results.	Godley 2023
	Not painful for patients; least invasive	Not true germline	
	High DNA yield		
Fingernails and toenails	Noninvasive	DNA extraction from nails needs additional sample processing.	Kakadia et al. 2018
		Low DNA yield	

Which genetic testing methods?

- The selection depends on **regulatory aspects, costs, and availability**.
- targeted gene NGS **panels, WES, WGS**.
- NHS: the implementation of WGS as a standard care practice for all patients with acute leukemia (A paired tumor and germline WGS, which facilitates identification of a greater number of germline variants) (Capturing).
- Because some germline predisposition alleles exist in noncoding regions, such as **promoters (e.g., ANKRD26**), **deeply intronic enhancers (e.g., GATA2)**, and **RNA encoding genes (e.g., TERC**), these regions need to be included in these assays, which may require augmentation through specific probes. The ever-expanding gene list complicates clinical testing.

Con…

- In myeloid neoplasms with DDX41 variants, 85% are germline and >95% of the truncating alleles are germline variants. Among DDX41 variants, the c.3G>A allele (truncated protein) is often detected as a germline variant.

In different ethnic groups: Japanese and Korean individuals are enriched with c.1496dup, whereas in Northern European c.3G>A and c.415_418dup are more common (2023).

- The presence of **multiple DDX41 variants**, especially those with high VAF, suggests a germline mutation.

Con…

- In the case of **RUNX1**, the **same variants** have been identified in both **somatic and germline** settings within HMs, highlighting the challenge of determining when to use germline confirmation.

Germline RUNX1 variants are distributed throughout the gene, necessitating sequencing the entire gene. These variants include missense, nonsense, frameshift, and whole-exon deletions or duplications.

- Germline **ANKRD26** variants are located in the **5'UTR** of c.-116 to c.-134, leading to **overexpression** of ANKRD26 owing to the failure of regulation by transcription factors RUNX1 and FLI1; thus, this region should be included in analyses

- In Shwachman Diamond syndrome (SDS): Biallelic pathogenic variants of SBDS (within exon 2, c.258+2 T>C and c.183_184delinsCT). Challenges: **SBDSP1 pseudogene** (shares 97% seq), **cis or trans.**

Gene	Predisposition to hematopoietic malignancies	Clinical features observed	Predisposition to solid tumors?	Reference(s)
ANKRD26	AML, CML, and MDS	Platelet dysfunction and thrombocytopenia	Yes	Sullivan et al. 2022
ATM	CLL, lymphoid leukemias, and lymphomas	Biallelic disruption causes ataxia telangiectasia	Yes	Stubbins et al. 2022
BRCA1	HMs	NR	Yes	Li et al. 2022, Stubbins et al. 2024
BRCA2	HMs	NR	Yes	
CEBPA	AML	Patients whose AMLs are treated with chemotherapy only are at risk for second independent AMLs in the future.	NR	Yuan et al. 2023
CHEK2	HMs	NR	Yes	Stolarova et al. 2020, Stubbins et al. 2022
DDX41	MMs	Older age of MM male > female	Yes, in some families, with unclear relationship to the *DDX41* variant	Makishima et al. 2023a,b
ETV6	B cell ALL > MMs	Thrombocytopenia, hyperchromatic megakaryocytes	Yes	Feurstein & Godley 2017, Wagener et al. 2023
GATA2	MMs	Cytopenias, HPV, EBV, pulmonary complications, reproductive issues	NR	Rajput & Arnold 2023, Santiago et al. 2023
IKZF1	CVID, ALL	High WBC count, not sensitive to glucocorticoid induction	NR	Wagener et al. 2023
PAX5	B cell ALL	NR	NR	Fouad & Eid 2023, Wagener et al. 2023
RUNX1	MM > T cell ALL	Platelet dysfunction and thrombocytopenia	Yes	Wagener et al. 2023
SAMD9	MMs	MIRAGE syndrome	NR	Narumi 2022, Rudelius et al. 2023
SAMD9L	MMs	Cerebellar ataxia, pancytopenia	NR	Narumi 2022
TP53	Hypodiploid ALL and MMs	NR	Yes	Abel et al. 2023

	Gene	Predisposition to solid tumors?	Reference(s)
Myeloid	*SAMD9*	NR	Narumi 2022
	SAMD9L	NR	Narumi 2022, Rudelius et al. 2023
	ARID1A	NR	Andrades et al. 2023
	CBL	NR	Hecht et al. 2022
	DIS3	NR	Ohguchi & Ohguchi 2023
	JAK2	NR	Tefferi 2021, Tefferi & Barbui 2020
	KDM1A	Yes	Zhang et al. 2021
	MBD4	Yes	Palles et al. 2022
	MPL	NR	Passamonti & Mora 2023
	NF1	Yes	Peduto et al. 2023
	PTPN11	Yes	Christofides et al. 2023
	RBBP6	Yes	Bi et al. 2021, Wang et al. 2020
	USP45	NR	Kraft & Godley 2020
Lymphoid	*CASP10*	Yes	Palmisani et al. 2023
	CD27	NR	Flieswasser et al. 2022, Palmisani et al. 2023
	CD70	Yes	Flieswasser et al. 2022
	CTLA4	NR	López-Nevado et al. 2021
	ITK	NR	
	MAGT1	NR	Doi & Okada 2020
	MRTFA	Yes	Reed et al. 2021
	TNFRSF9	NR	Claus et al. 2023
	UNC13D	NR	Sadeghi et al. 2022
Myeloid/lymphoid	*BLM*	Yes	Ababou 2021
	CSF3R	NR	Guastafierro et al. 2023, Szuber & Tefferi 2021
	RECQL4	NR	Luong & Bernstein 2021
	TET2	NR	Belizaire et al. 2023
	TP53	Yes	Abel et al. 2023, George et al. 2021, R. Kim et al. 2023, Saiki & Ogawa 2023
	SH2B3	Yes	Beghini et al. 2022
	WAS	Yes	Hsu 2023
Bone marrow failure	*DNAJC21*	NR	Feurstein 2023
	ERCC6L2	NR	Armes et al. 2022, Baccelli et al. 2023, Douglas et al. 2019, Feurstein 2023, Hakkarainen et al. 2023, Shabanova et al. 2018
	DCLRE1B	NR	Kermasson et al. 2022
	SBDS	NR	Kawashima et al. 2023, Spinetti et al. 2022
	EFL1	NR	Kawashima et al. 2023
	MECOM	Yes	Lozano Chinga et al. 2023
	NAF1	Yes	Batista et al. 2022
	NPM1	NR	Khan & Gartel 2022
	RTEL1	NR	Grill & Nandakumar 2021
	SRP72	NR	Faoro & Ataide 2021, Lovatel et al. 2023

	Gene	Predisposition to solid tumors?	Reference(s)
Immunodeficiency syndromes	*BTK*	Yes	Ahn & Brown 2021, Shadman 2023
	CARD11	NR	Zhao et al. 2022
	CD40LG	NR	Tang et al. 2021
	CTPS1	Yes	Asnagli et al. 2023
	DOCK8	NR	Zhang et al. 2022
	NBN	NR	Otahalova et al. 2023
	PGM3	Yes	Fallahi et al. 2022
	PIK3CD	Yes	Ames et al. 2023
	PTEN	Yes	Álvarez-Garcia et al. 2019
	RASGRP1	NR	López-Nevado et al. 2021
Telomere biology disorder genes	*TERT*	Yes	Byrjalsen et al. 2023, Kam et al. 2021
	TERC	Yes	
	DKC1	Yes	
	ZCCHC8	Yes	Kam et al. 2021, Savage & Niewisch 1993
	PARN	Yes	Batista et al. 2022, Kam et al. 2021
	CTC1	Yes	Grill & Nandakumar 2021
Amyloidosis	*TTR*	NR	Ioannou et al. 2023
	APOA1	NR	Jeraj et al. 2021
	APOA2	NR	
	CST3	NR	Jiang et al. 2020
	FGA	NR	Chyra Kufova et al. 2018
	GSN	NR	Potrč et al. 2021
	LYZ	NR	Chyra Kufova et al. 2018

Abbreviation: NR, not reported.


natologic Malignancies
Germline Predisposition in Hematologic Malignancies: Testing, Management, and Implications
Lucy A. Godley, MD, PhD[1]; Courtney D. DiNardo, MD, MSCE[2]; and Kelly Bolton, MD, PhD[3]
DOI https://doi.org/10.1200/EDBK_432218
OVERVIEW
Although numerous barriers for clinical germline cancer predisposition testing exist, the increasing recognition of deleterious germline DNA variants contributing to myeloid malignancy risk is yielding steady improvements in referrals for testing and testing availability. Many germline predisposition alleles are common in populations, and the increasing number of recognized disorders makes inherited myeloid malignancy risk an entity worthy of consideration for all patients regardless of age at diagnosis. Germline testing is facilitated by obtaining DNA from cultured skin fibroblasts or hair bulbs, and cascade testing is easily performed via buccal swab, saliva, or blood. Increasingly as diagnostic criteria and clinical management guidelines include germline myeloid malignancy predisposition, insurance companies recognize the value of testing and provide coverage. Once an individual is recognized to have a deleterious germline variant that confers risk for myeloid malignancies, a personalized cancer surveillance plan can be developed that incorporates screening for other cancer risk outside of the hematopoietic system and/or other organ pathology. The future may also include monitoring the development of clonal hematopoiesis, which is common for many of these cancer risk disorders and/or inclusion of strategies to delay or prevent progression to overt myeloid malignancy. As research continues to identify new myeloid predisposition disorders, we may soon recommend testing for these conditions for all patients diagnosed with a myeloid predisposition condition.
Accepted April 3, 2024
Published May 20, 2024
Am Soc Clin Oncol Educ Book 44:e432218
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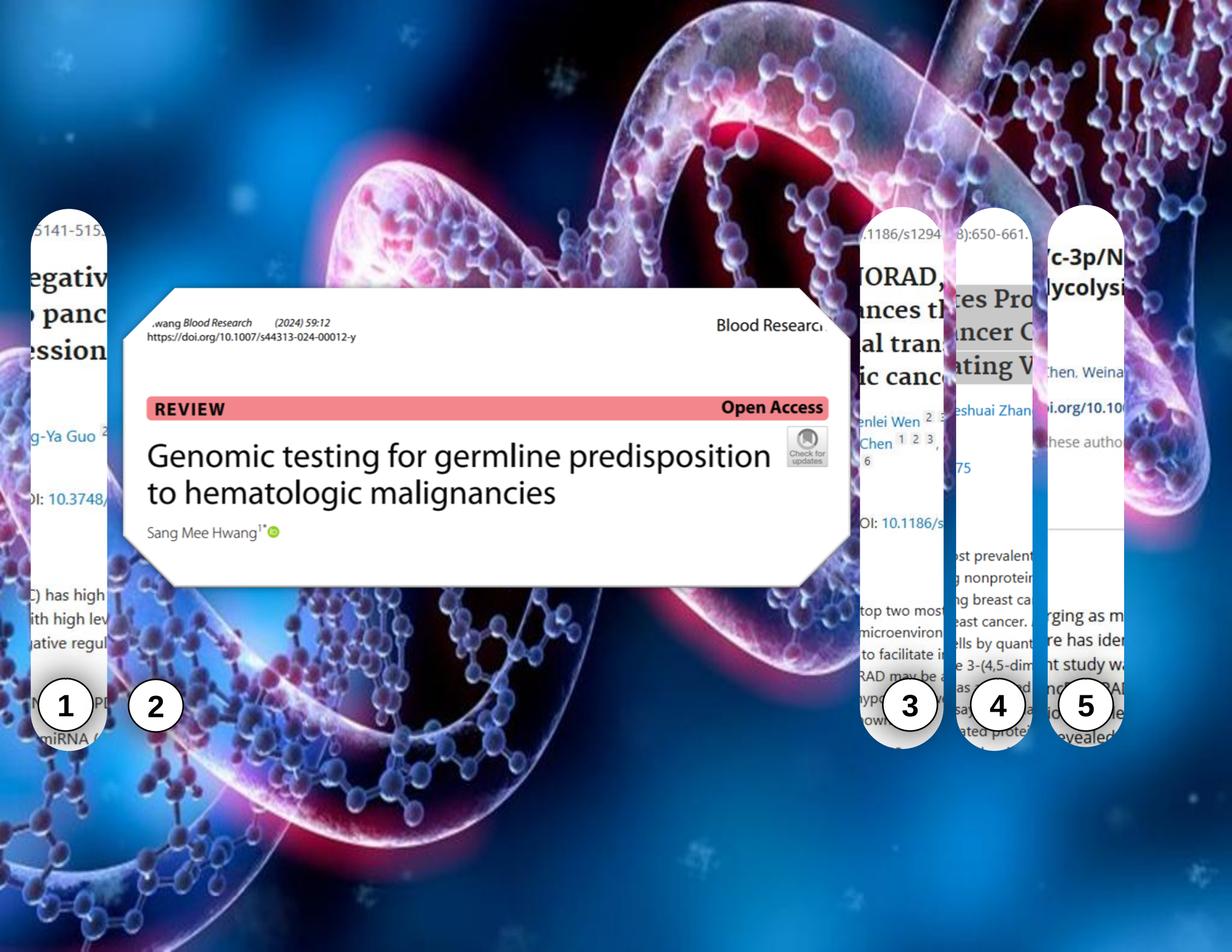
Hwang Blood Research (2024) 59:12
https://doi.org/10.1007/s44313-024-00012-y
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REVIEW
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Genomic testing for germline predisposition to hematologic malignancies
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ANNUAL REVIEWS
Annual Review of Cancer Biology
Germline Predisposition to Hematopoietic Malignancies: An Overview
Yogameenakshi Haribabu, Emma Bhote, and Lucy A. Godley
Division of Hematology/Oncology, Department of Medicine and Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Chicago, Illinois, USA; email: lucy.godley@northwestern.edu
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Annu. Rev. Cancer Biol. 2024. 8:309–29
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Keywords
germline, cancer predisposition, hematopoietic malignancies, bone marro
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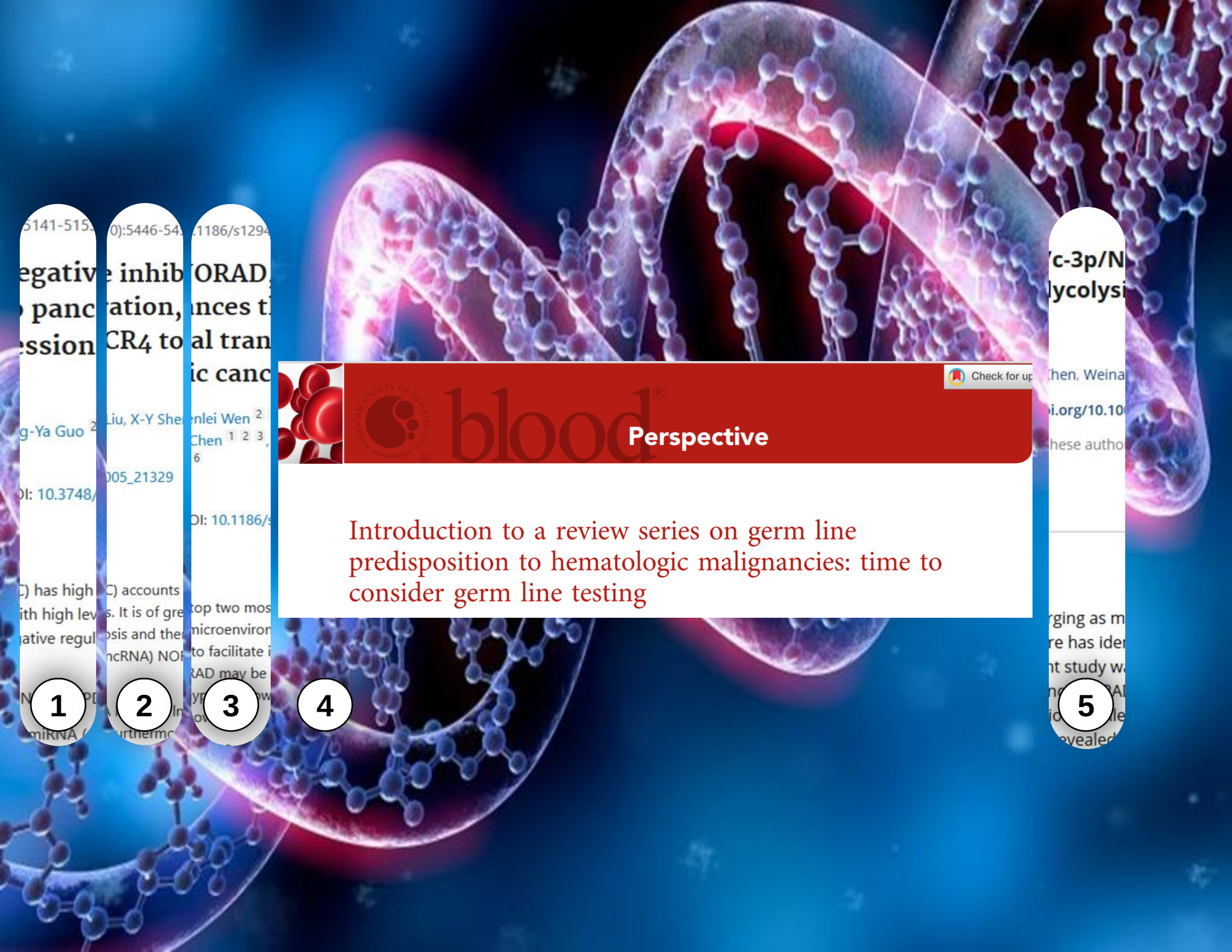
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Introduction to a review series on germ line predisposition to hematologic malignancies: time to consider germ line testing
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...d: 29 November 2022 | Accepted: 17 January 2023

...OI: 10.1111/bjh.18675

BJHaem

GUIDELINE

Germline predisposition to haematological malignancies: Best practice consensus guidelines from the UK Cancer Genetics Group (UKCGG), CanGene-CanVar and the NHS England Haematological Oncology Working Group

Beverley Speight[1] | Helen Hanson[2,3] | Clare Turnbull[3,4] | Steven Hardy[5] | James Drummond[1] | Jamshid Khorashad[3] | Christopher Wragg[6] | Paula Page[7] | Nicholas W. Parkin[8] | Ana Rio-Machin[9] | Jude Fitzgibbon[9] | Austin Gladston Kulasekararaj[10,11,12] | Angela Hamblin[13] | Polly Talley[14,15] | Terri P. McVeigh[3,4] | Katie Snape[2,10] | on behalf of Consensus Meeting Attendees

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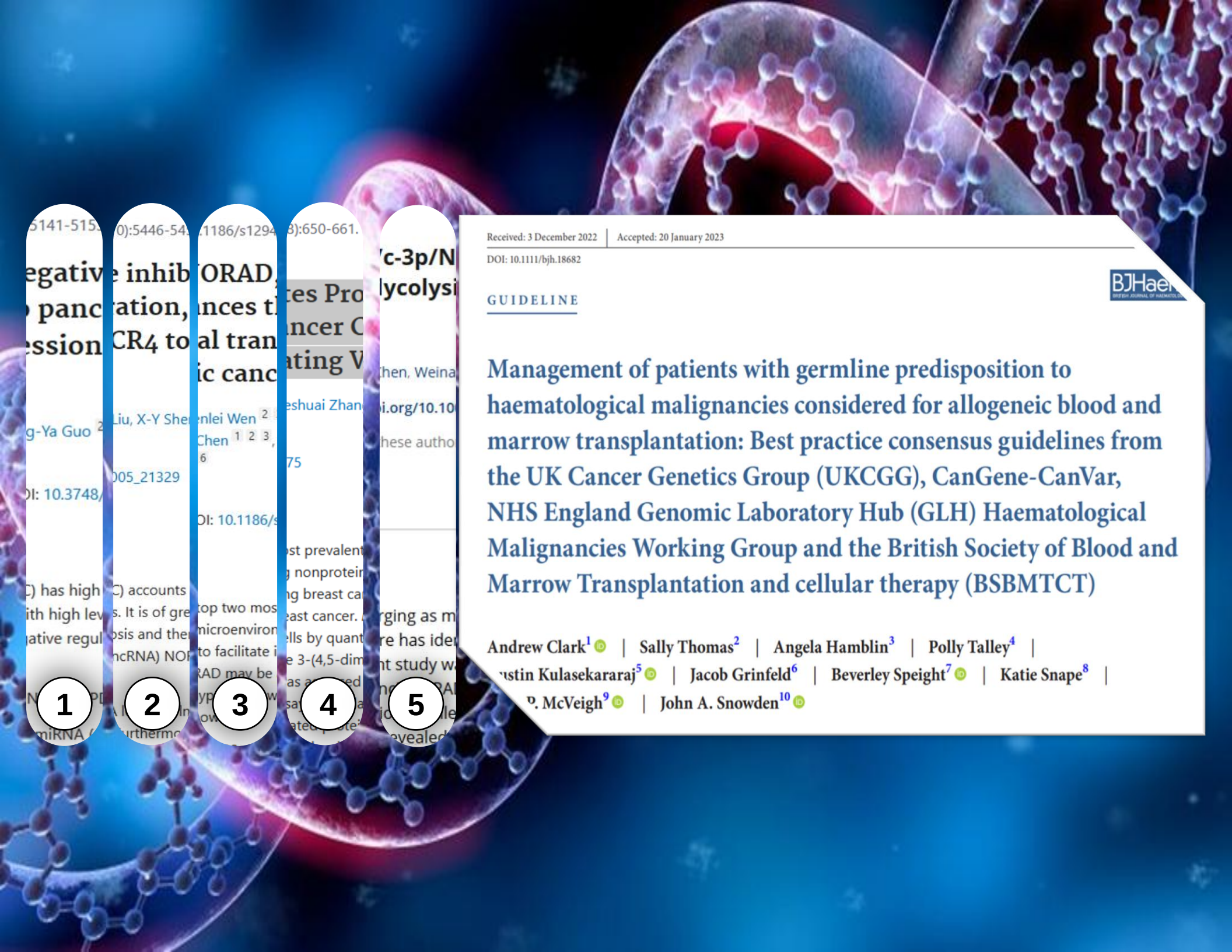
[9]Centre for Genomics and Computational Biology, Barts Cancer Institute, Queen Mary University of London, London, UK
[10]King's College Hospital NHS Foundation Trust, London, UK
[11]National Institute for Health and Care Research and Wellcome King's Research Facility, London, UK
[12]King's College London, London, UK
[13]Oxford University Hospitals NHS Foundation Trust and Central and South Genomic Laboratory Hub, Oxford, UK
[14]Genomics Unit, NHS UK and NHS Improvement, Leeds, UK
[15]North East and Yorkshire Genomic Laboratory Hub, Leeds, UK



Received: 3 December 2022 | Accepted: 20 January 2023

DOI: 10.1111/bjh.18682

GUIDELINE

Management of patients with germline predisposition to haematological malignancies considered for allogeneic blood and marrow transplantation: Best practice consensus guidelines from the UK Cancer Genetics Group (UKCGG), CanGene-CanVar, NHS England Genomic Laboratory Hub (GLH) Haematological Malignancies Working Group and the British Society of Blood and Marrow Transplantation and cellular therapy (BSBMTCT)

Andrew Clark[1] | Sally Thomas[2] | Angela Hamblin[3] | Polly Talley[4] | ustin Kulasekararaj[5] | Jacob Grinfeld[6] | Beverley Speight[7] | Katie Snape[8] | P. McVeigh[9] | John A. Snowden[10]

Donor Selection

Donor derived leukemia :

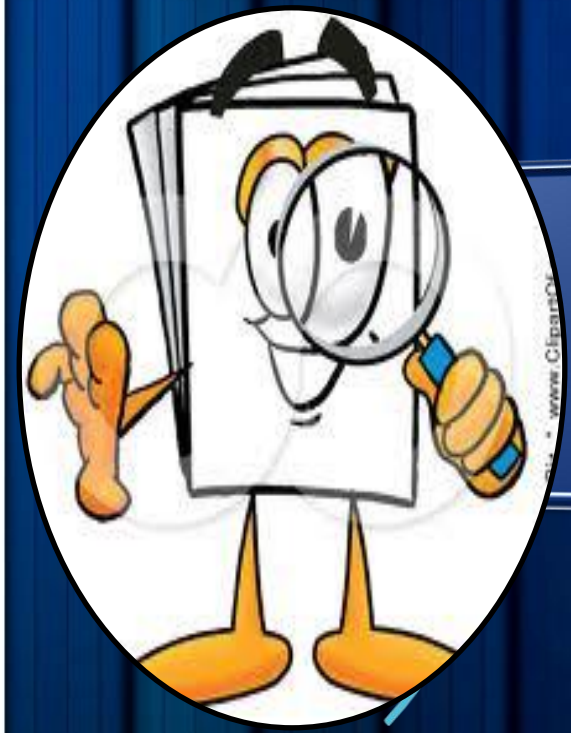
while one significant **motivator to enroll** in unrelated donor **registries** is the presence of leukemia in a family member.

- These risks are primarily mitigated by using **questionnaires** as universal screening for germline predisposition syndromes is not available for **volunteer unrelated donors**.
- However, in the future, given the availability of high-throughput HLA-typing and sequencing, the **feasibility of screening for germline** predisposition may be explored, particularly as the **safety of mobilization** using **GCSF** in the context of an occult germline mutation is **unknown.**

Donor Selection

Recommendations

- *It is important to avoid inadvertently using a carrier relative as a donor. This requires a high index of suspicion, and appropriate, timely, targeted testing of patients and their potential family donors.*[2,3]
- *Where the predictive testing of a germline variant in potential donor relatives has been undertaken urgently to inform BMT decisions, matched relatives shown NOT to carry the variant would usually be prioritised over matched carrier relatives.*[1]
- *Where predictive testing of a germline variant in potential donor relatives has been undertaken urgently to inform BMT decisions, a relative shown to be a carrier would not usually be considered as a potential donor unless other options were limited.*[1]
- *If concerned about a strong family history/syndromic features in the absence of a confirmed genetic diagnosis, it would be best practice to discuss in the MDT meeting to document the history and decide whether related donors should be prioritised above unrelated donors.*[1]
- *Where all related matched donors are either carriers or decline testing, careful assessment of risks and benefits of an unrelated donor versus a carrier family member/untested family member requires discussion at a MDT meeting with access to expert opinion and consideration on a gene-specific basis.*[1]



THANKS

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