Guidance for the Management of possible Germline Variants Identified During Routine Genomic Testing in Haematology

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Prepared by: Tom Coats, Andrew Mumford, Jonathan Miller, Sarah Westbury, Alan Donaldson, Claire Lentaigne, Matilda Bradford and Ruth Cleaver.

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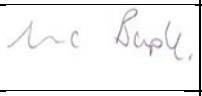
The following people must approve this document:

Name	Signature	Title	Date	Version
Emma Baple		Medical Director SWGLH	14/11/2023	2.0
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1. Executive Summary

Standard of care genomic testing may identify pathological variants in genes that ***may*** be germline variants, and therefore relevant to the patient and their wider family. Only testing with a germline or constitutional sample can confirm if a variant is in the germline. Whether to proceed with germline testing requires careful discussion with the patient ***before*** the germline sample is taken, as this will have implications for other family members. This document will provide a basic overview to help guide which patients could be considered for **confirmatory germline testing**.

Should a germline variant be confirmed, the haematologist is advised to refer the patient to their local Clinical Genetics (CG) service to facilitate counselling and, where appropriate, family testing. The referring haematologist's responsibility is to advise the CG team on the next steps for any family members identified with the inherited variant. There are currently no national clinical practice guidelines for surveillance for the majority of genes relevant to haematological malignancy. Work is being undertaken in this area by a national collaboration of key stakeholders (see section 10). The regional acute leukaemia advisory panel can support discussions about this and can be consulted prior to, or after, confirmatory germline testing.

2. Introduction

There is an increased use of genomic testing in the routine work-up of haematological malignancies, including the targeted testing of a panel of relevant genes. As a result, genetic variants may be identified in blood or bone marrow samples that are possible germline variants. To maximise the likelihood of identification of actionable germline findings, the laboratory will only highlight a potential germline finding following somatic testing of:

- High actionability haematological cancer susceptibility genes sequenced for their somatic significance, i.e. *DDX41, CEBPA, RUNX1* and *TP53* in younger patients
- Variants with a variant allele frequency (VAF) suggestive of a potential germline origin, i.e. VAF >30%

This will be flagged on the NGS panel reports, e.g. "*The DDX41 variant was detected at a frequency indicating possible germline origin. If there is a clinical suspicion of an underlying germline condition, then a germline skin, or remission blood sample will be required for constitutional analysis. If a germline origin is proven, this finding may have implications for the patient and their wider family".*

Please note that any germline focussed analysis undertaken by the GLH will ensure that only genetic variants likely to be biologically pertinent are flagged e.g. pathogenic or likely pathogenic variants.

Confirmation of whether or not an identified genetic variant is a germline variant requires additional tissue (usually either a skin biopsy or a remission blood sample). In some circumstances, confirmation of a germline finding may not be appropriate and a careful discussion with the patient is required *before* confirmatory samples are sent. In the absence

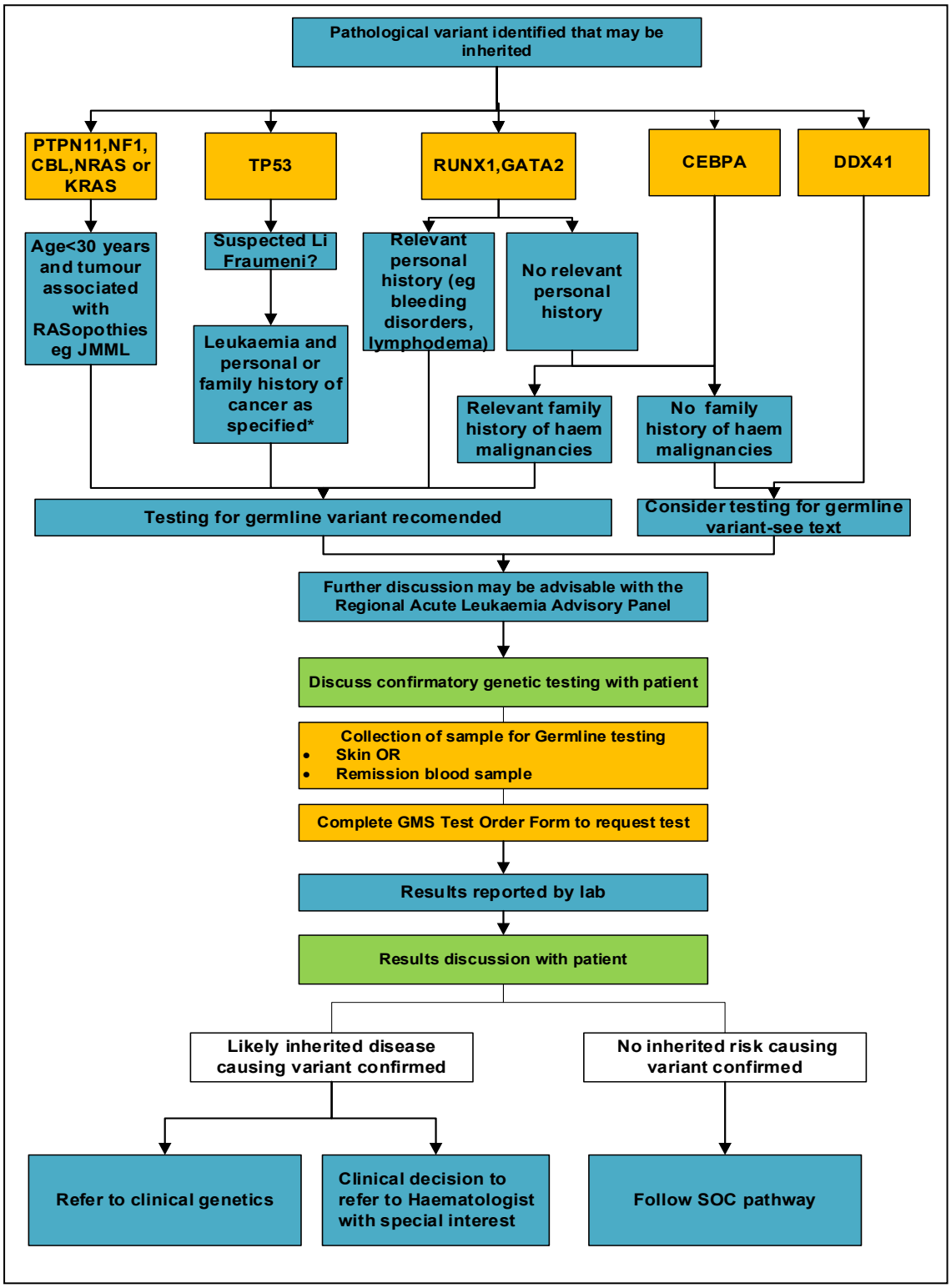
of any national guidance on this topic, this document will outline some considerations and practicalities for health care practitioners in the South West region.

3. Germline variants relevant to haematological malignancy

ESMO guidelines [Mandelker 2019] have defined a set of high actionability cancer susceptibility genes that, when apparent in the context of a related tumour, should prompt consideration of a potential germline origin. These are listed below with some comments taken from the *Association for Clinical Genomic Science (ACGS) draft document*:

- *PTPN11*, *NF1*, *CBL*, *NRAS* and *KRAS* - restrict germline analysis to patients under 30 years of age and for tumours associated with RASopathies, for example JMML.
- *TP53* – in Li Fraumeni syndrome (LFS) associated tumours. As reflected in the NHS England National Genomic Test Directory criteria, LFS can involve a wide spectrum of tumours, and defines the following as Li Fraumeni associated cancers; sarcoma of bone/soft tissue, breast cancer, central nervous system tumours, and adrenocortical cancer.
- Confirmatory germline testing of a somatic TP53 variant should be considered in a patient with:
  - Leukaemia diagnosed before the age of 46 and personal history of another LFS tumour (diagnosed before age of 46)
  - Leukaemia diagnosed at any age and personal history of two LFS tumours (both diagnosed under age 46)
  - Leukaemia and at least one first or second-degree relative with an LFS-related tumour (one case ≤46 years, the other ≤56years. If one is breast cancer, limit to diagnosis ≤35years)
  - Leukaemia and ≥2 first/second degree family members with early onset (≤45years) LFS-related cancers
- *ANKRD26*, *CEBPA*, *DDX41*, *ETV6*, *GATA2*, *RUNX1* – as listed in the 2016 WHO Classification under the myeloid malignancies with germline disposition. The lifetime risk for myeloid malignancies varies by gene. *ANKRD26* and *ETV6* are not routinely reported on in the myeloid NGS panels, so won't be discussed further here. The ELN guidelines on managing AML recommend that physicians take a thorough patient and family history to assess for typical signs and symptoms of known syndromes, including data on malignancies and previous bleeding episodes.

Further information is given on these genes in the section below.



*3.1.1. *Leukemia and another LFS tumour (both diagnosed before 46) OR Leukemeia and at least one first or second degree relative with a LFS related tumour (one occuring at 46 or under, the other at 56 or under) where LFS associated tumours = sarcoma of bone/soft tissue, breast cancer, CNS tumours, adenocortical cancer or any other childhood cancer (occurring ≤ 18 years)*

Figure 1: Schema to guide germline testing in a patient with a possible germline variant identified in routine clinical testing.

4. Further guidance on when to perform testing for germline variants in CEBPA, DDX41, GATA2 and RUNX1

For variants identified in *CEBPA*, *DDX41*, *GATA2*, *RUNX1* in the presence of a strong personal history (i.e. bleeding disorder, lymphodema etc.) or family history of haematological malignancies, it is recommended to discuss confirmatory testing with the patient. This would be followed by a referral to Clinical Genetics in the event of confirming a germline variant. The lifetime risk for myeloid malignancies varies by gene:

- *CEBPA* and GATA2 deficiency syndrome >80%,
- *RUNX1* 45%,
- *DDX41* unknown, probably high but mostly in older age

By comparison, the lifetime risk for AML and myeloid malignancies in the general population is approximately 0.5% and 2% respectively. A large Swedish registry study indicates that if a first-degree relative has AML, there is a 1.5-fold increased risk of developing both AML or a myeloid malignancy, i.e. 0.75% and 3% lifetime risk. Therefore, identifying a well-described gene variant can represent a significant increase in the risks of a family member developing a haematological cancer.

It is important to note that the exact site, or loci, within the gene where the variant occurs may also impact the likelihood of that variant being associated with an inherited predisposition to leukaemia. Some loci of familial associated variants also occur as somatic variants in sporadic AML/MDS, whereas others are enriched or exclusive to inherited forms of myeloid malignancy. The GLH lab will have done a preliminary search of relevant databases prior to issuing a report to filter out variants in candidate genes that are highly unlikely to be associated with an inherited predisposition.

For patients with no personal or family history to suggest a hereditary predisposition, a **referral to clinical genetics may still be appropriate**. Discussion with the Regional Acute Leukaemia Advisory Panel (RALAP) is recommended.

Consideration should also be given to the presence of germline variants in *DDX41*, as this is associated with a late-onset presentation of myeloid malignancies (MDS and AML), so **early identification may not be desirable**.

All index patients should be counselled that there are no known proven interventions regarding monitoring family members should they have a germline predisposition to haematological malignancy e.g. regular FBC or BM testing. More details on potential strategies below.

5. What to do if a possible germline variant is identified in routine, standard of care testing?

Please see *figure1* for an overview.
Upon identification of a possible germline variant it is recommended that, if not already done so, a careful personal and family history is taken with regards to any relevant medical history or prior malignancies. A discussion with the Regional Acute Leukaemia Advisory Panel (RALAP) – consisting of haematologists with a special interest in leukaemia, clinical

geneticists and clinical scientists - may be advisable prior to arranging confirmatory germline testing. This is a relatively new and expanding area in haematology so, until local expertise develops, discussions to establish the relevant considerations including pros and cons may be desirable.

As per figure 1, a recommendation to proceed to confirmatory testing is more clear cut in certain scenarios e.g. RASopathy with *NRAS* variant in a young patient, or *RUNX1* in a patient with a personal history of bleeding. The decision is potentially more complex for genes such as *TP53* where the broad range of associated cancer risks are very significant for the patient and their family members. Discussion with the RALAP prior to testing may be desirable to support the haematologist and to discuss any issues arising from the case.

If the patient is being worked up for a possible allogeneic stem cell transplant, and family members are being considered as potential donors, then early discussion with the transplant centre and clinical genetics is recommended.

Ultimately, it is the responsibility of the haematologist to inform the patient of the possibility of a germline genetic variant and arrange onward testing if appropriate.

6. How to confirm a germline variant?

A germline variant can be confirmed by comparing the tumour sample to a non-affected tissue. This can be done by taking a skin punch biopsy (see appendix 1 on how to do this). Alternatively, take a remission blood sample (providing the patient has not had an allogeneic stem cell transplantation). Please send a sample with a BHODS form and email SWGLHhaemonc@nbt.nhs.uk to notify the lab of the sample request.

7. When to involve Clinical Genetics?

A referral to Clinical Genetics is recommended if germline testing has confirmed the presence of a germline variant.
Cases can be referred to the RALAP to facilitate a discussion with Clinical Genetics prior to, or following, confirmatory testing.

8. What support can be expected from the Clinical Genetics service for patients with a germline variant?

8.1. For affected individuals with a germline variant clinical genetics will discuss the implications for the patient as well as the risks to family members. Discussions may include advice about disease risk, testing for relatives, screening/management, family support and reproductive options.

8.2. For unaffected individuals where a germline variant has been identified in a family member, clinical genetics will discuss the implications of this variant, and support the person in deciding about predictive genetic testing. If the genetic variant is found following a predictive test, they will be given appropriate screening advice with the input of haematology, where needed, and taking into account national guidance as this becomes available.

Please note: unaffected relatives should not be offered genetic testing outside of Clinical Genetics.

9. Monitoring and advice for family members with an inherited germline variant

Where an inherited genetic variant is found following a predictive test, Clinical Genetics will offer referral of appropriate individuals to their local haematology service for opinion/assessment. From there, the recommendation of this document would be to consider the following: (NB this is not an exhaustive list, nor evidence-based):

i) provision of advice about symptom awareness
ii) clinical assessment for any related clinical features (bleeding history, lymphoedema etc.)
iii) baseline BM assessment
iv) regular (i.e. 6-12 monthly) FBC monitoring – undertaken in primary or secondary care
v) myeloid gene panel on peripheral blood periodically to look for the acquisition of new myeloid mutations to suggest disease evolution – this indication is not necessarily funded currently but could be discussed with a local GLH
vi) onward referral to a tertiary centre with a specialist interest in inherited myeloid disorders

More information can be found by reviewing this "How I treat" article. (Blood 2016;1800-13) and by reviewing the UKCGG/CanGene-CanVar consensus workshop Apr 2022 (see next section)

10. Future work and updates

A national workshop was convened in April 2022 by the UK Cancer Genetics Group (UKCGG), in collaboration with CanGene-CanVar and NHS England Haematological Oncology Malignancies working group. This workshop set out to address the lack of national guidance on the investigation and management of inherited leukaemia predisposition variants. Ref: Management-of-patients-with-germline-predisposition-to-haematological-malignancies-1-2

Further work has been completed through this collaboration to develop gene-specific guidelines and standardised patient information. See; UKCGG Resources for clinicians and for carriers of constitutional pathogenic variants associated with haematological malignancy

Regionally, the SW Genomic Medicine Service is running a project to support clinicians and their patients with genetic variants that may lead to an inherited predisposition to leukaemia to receive consistent and coordinated support and advice. The project objectives are

- Determine geographical variation and barriers to access to germline testing pathways across all relevant providers.
- Develop and disseminate germline testing policy adapted for SW practice.
- Embed ubiquitous testing for germline leukaemia predisposition variants across the geography, linked to mainstream pathways
- Demonstrate clinical impact of access to personalised/stratified care following demonstration of leukaemia susceptibility

Outputs from this project and the national working group will be shared/updated as it evolves.

11. Germline variants identified during whole-genome sequencing

Variants in the genes listed above may be identified and established as a germline variant when patients are tested via Whole Genome Sequencing (WGS) with paired constitutional and tumour samples. If this happens, the same process regarding the involvement of Clinical Genetics should be followed. Variants of unknown significance (as opposed to pathogenic/likely pathogenic variants which are discussed in this document) will be marked as such in the WGS result. Attendance at the RALAP meeting where the WGS results will be reviewed and disseminated is recommended for further input for patients with germline variants identified through WGS.

For any issues regarding this guidance document, the SW GLH haemato-oncology lead (Dr Tom Coats, thomas.coats@nhs.net, 07815091735) can be contacted to discuss.

12. References

- Nordic Guidelines for Germline Predisposition to Myeloid Neoplasms in Adults: Recommendations for Genetic Diagnosis, Clinical Management and Follow-up. Baliakas et al. HemSphere 2019.
- Germline-focussed analysis of tumour-only sequencing: recommendations from the ESMO Precision Medicine Working Group Ann Oncol. 2019
- Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Dohner et al. Blood 2017.
- Acute myeloid leukaemia with genetic abnormalities. In: Swerdlow et al, WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Update to 4th Edition. Lyon, France. In press.
- How I diagnose and manage individuals at risk for inherited myeloid malignancies. Churpek et al. Blood. 2016:1800-13
- The complex genetic landscape of familial MDS and AML reveals pathogenic germline variants. Rio-Machin et al. Nat Commun: 2020: 1044
- Familial risks of acute myeloid leukaemia, myelodysplastic syndromes, and myeloproliferative neoplasms. Sud et al. Blood 2018: 973-6
- Myeloid malignancies in the real-world: Occurrence, progression and survival in the UK's population-based Haematological Malignancy Research Network 2004-15. Roman et al. Cancer Epidmiol. 2016: 186-98

Appendix 1 Performing the punch skin biopsy

i) Ensure sterile field and handwashing as appropriate for a locally invasive procedure.

ii) The biopsy area should be cleaned for 30s with an alcohol-based cleansing solution such as Chloraprep® and allowed to dry for another 30s.

iii) Infiltrate local anaesthetic such as Lidocaine 1% and/or apply topical local anaesthetic such as Ametop® or Emla® if required.

iv) Stretch the skin perpendicular to normal relaxation lines and introduce a 4mm disposable punch biopsy blade firmly at a perpendicular angle to the anaesthetised skin. Rotate through 45 degrees, repeatedly carrying the blade through the skin through to the subcutis. The biopsy guard on the sterile punch biopsy will prevent deeper penetration.

v) Withdraw the sterile punch biopsy whilst applying pressure on the puncture site with a non-woven swab. This should release the skin specimen.

vi) If the sample is not released, use plastic disposable forceps and disposable scalpel or sterile scissors to release the sample from the biopsy site.

vii) Place the specimen in a pre-labelled empty universal container containing sterile saline. **Do not put the specimen in formalin.**

viii) Apply continuous pressure to biopsy site for three to five minutes or until bleeding stops.

Aftercare of the biopsy site

Once bleeding has stopped, apply Steri-strips® or similar in a 'star' pattern. Sutures are not routinely required. Apply a dry dressing such as Cutiplast® or Op-site®. Consider a low-adherent dressing such as Mepitel® if the surrounding skin is fragile.

The site should be kept dry and left untouched for 48 hours, then the dressing removed. Once skin edges have sealed, bathing or showering is acceptable.