Germline Predisposition to Haematological Malignancies
Consensus guidelines meeting 28-29th April 2022

Background reading document

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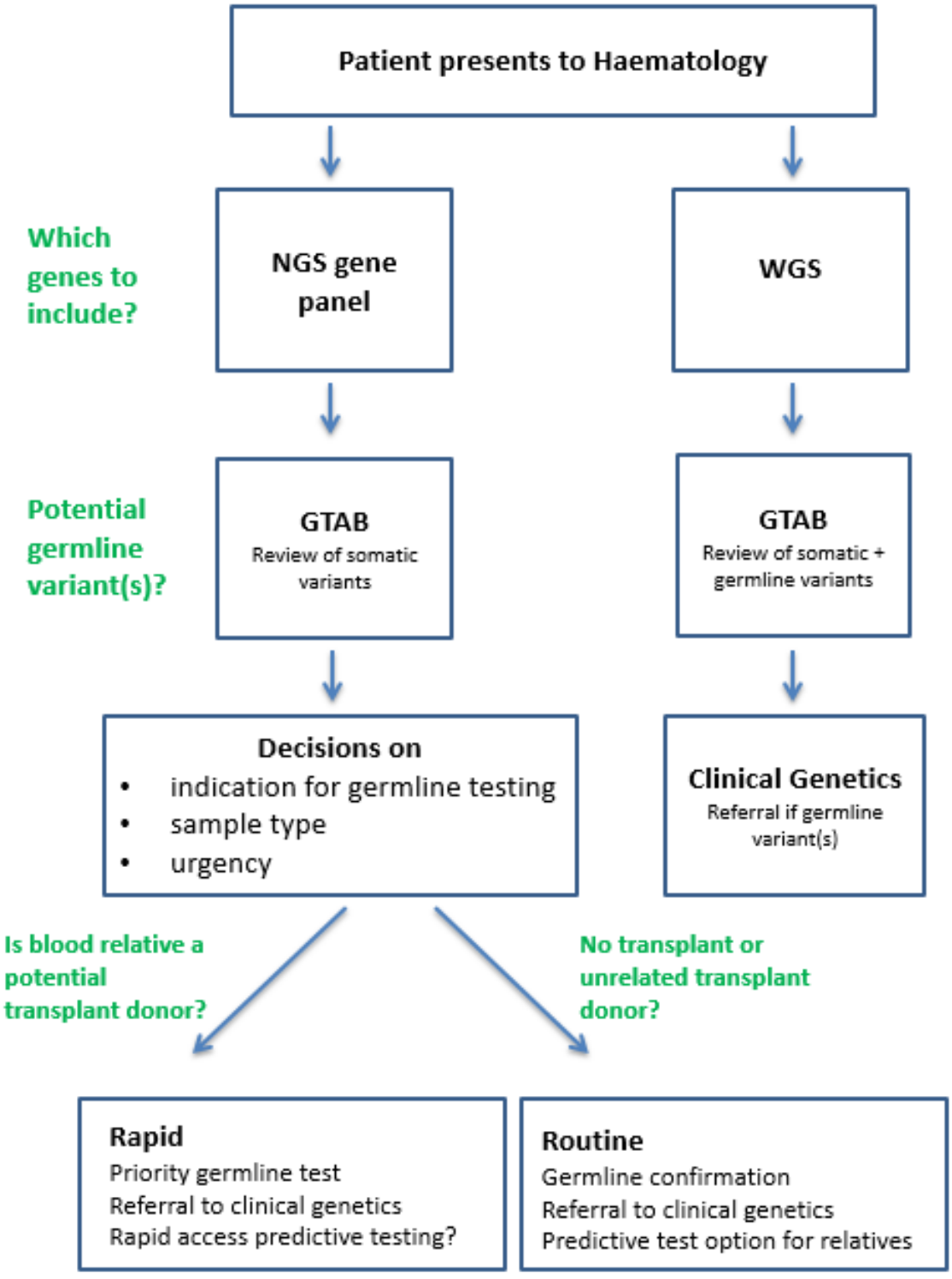
Introduction

Next generation sequencing (NGS) is now standard of care in the diagnostic work-up of patients with suspected haematological malignancies. In England, eligibility for testing is set by the National Genomic Test Directory (www.england.nhs.uk/publication/national-genomic-test-directories/). The identification of somatic gene variants can inform diagnosis, prognosis and therapeutic options. Somatic gene panels and Whole Genome Sequencing (WGS) can also identify individuals with potential or confirmed germline predisposition to haematological malignancy. Identifying these patients and setting up pathways for appropriate germline confirmation with the possibility of cascade predictive testing in family members is a relatively new area of expanding clinical and laboratory work. Current challenges within this include:

- Which genes and variants should be analysed and reported?
- What sample types are best used?
- What is the pathway for patients needing a clinical genetics referral?

The UK-CGG, CanGene-CanVar and the NHSE GLH Haematological Oncology Malignancies Working Group have convened these two half-day workshops to try to address these challenges. The aim of the meeting is to establish consensus guidelines on the clinical and laboratory pathways and the management of disease causing germline variation. Given this is a substantial topic, the meeting will focus on six genes: *DDX41, CEBPA, RUNX1, ANKRD26, ETV6, GATA2*. Hopefully any consensus reached will be applicable to an expanding number of other genes in this setting.

Figure 1. Flowchart highlighting current challenges



Current pathway challenges

From somatic detection to germline confirmation

In practice, bioinformatic pipelines undertaken through somatic testing strategies usually highlight variants within three different classifications according to the American College of Medical Genetics (ACMG) variant interpretation guidelines: Variants of Uncertain Significance (VUS) (Class 3), Likely Pathogenic (Class 4) and Pathogenic (Class 5). There are no definitive guidelines about when to proceed to germline testing after somatic panel testing for haematological malignancy. Traditionally, in UK Clinical Genetics services, a threshold of 10% for finding a likely pathogenic/pathogenic germline variant has been used for considering germline testing (NICE, 2013). Germline VUS are generally not considered clinically actionable, with the exception of rare cases when further investigations may provide additional evidence to upgrade them to likely pathogenic.

In 2020, the European Society for Medical Oncology (ESMO) Precision Medicine Working Group sought to generate guidelines on germline-focussed analysis of tumour sequencing data, indications for germline testing and patient information/consent. Their published recommendations were based on the somatic to germline conversion rate in paired sequencing data from more than 16,000 solid cancers (Mandelka *et al*, 2019). In summary, these guidelines advise that germline focussed analysis should be offered where a variant is predicted to be likely pathogenic/pathogenic in the germline setting, highly penetrant and found at a Variant Allele Frequency (VAF) of >30% for single nucleotide variants or >20% for small insertions or deletions. Caution was advised regarding variants in genes that are not proven through research to be of high penetrance, or variants in genes associated with disorders unrelated to the malignancy for which testing was done. Confirmation of VUS was not recommended. Research evidence on patient preferences indicates that a majority of cancer patients would prefer to know of incidental germline findings irrespective of whether the associated condition is preventable (Yushak *et al,* 2016).

Sample types

In the solid cancer setting, DNA extracted from peripheral blood leukocytes is a commonly used sample for germline testing. Alternative sources of germline DNA are saliva (in which the predominant DNA source is leukocytes) or a buccal epithelial scrape. In the Haematology cancer setting, blood and saliva are unsuitable due to the predominance of tumour DNA. Sample options in this setting are a skin biopsy, T-cell purified DNA sample from blood, buccal scrape or remission bone marrow sample. A summary table of some advantages and disadvantages of these is below. Other samples such as urine, finger nails and skin swabs have been shown to yield insufficient DNA for testing (Padron *et al*, 2018). Advantages and disadvantages of different sample types which could potentially be obtained for germline analysis are outlined in Table One.

Sample type	Advantages	Disadvantages
Skin biopsy	High quantity DNA source Cell culture option to increase yield	Invasive procedure May not be possible in all centres Requirement for culture to avoid contamination adds time delay to pathway If uncultured, chance of contamination by leukocyte-derived DNA
T-cell sorting	Easy accessible sample (blood)	Need specialist laboratory technique Cannot be done in all centres Possibility of early somatic haematopoietic mutations misdiagnosed as germline Gold standard involves expensive FACS sorting otherwise risk of contamination
Buccal scrape	Easy access sample Patients can self-sample at home	Contamination by leukocyte-derived DNA*
Remission bone marrow aspirate	Reduces the number of samples needing germline confirmation	May delay germline confirmation Not all patients have remission sampling Not suitable in urgent cases Possibility of residual clonal somatic haematopoietic mutations misdiagnosed as germline

*shown to be low if procedure optimised for epithelial cell recovery (Padron *et al*, 2018)

Table 1: Advantages/Limitations of sample types available for germline confirmation in patients with haematological malignancies

Consent and sample acquisition in germline testing pathways

The ESMO guidelines acknowledge that consent and sample storage for possible germline confirmation can be included ahead of somatic panel NGS, but is often a more staged approach, occurring after variant review and classification. Irrespective of timing, information provision and documented consent is conventional practice and a requirement prior to germline confirmatory testing. There are different approaches to confirmatory testing. There has been a general move towards mainstream germline testing in the solid cancer setting, with a referral for genetic counselling following confirmation of a germline pathogenic variant (Flaum *et al*, 2020). Survey-based qualitative research evidence in a separate study of nearly 1000 solid cancer patients showed high acceptability and satisfaction of a mainstream genetic testing model (Hamilton *et al*, 2021). Suggested models of care in the haematological setting also reflect the need for patient information and informed consent at various stages, with a requirement for formal genetic counselling around confirmation of a germline pathogenic variant (Godley & Shimamura, 2017; Tawana *et al*, 2018; Baliakas *et al*, 2019).

Donor selection

If treatment of haematological malignancy is to involve allogeneic hematopoietic stem cell transplantation, donor matching warrants consideration of any known germline predisposition (Godley & Shimamura, 2017). If an asymptomatic related donor is being considered, predictive testing for the known variant should be offered. Donor-derived leukaemias have been reported after transplant from a relative who was a carrier of a familial germline pathogenic variant in *CEBPA* (Xiao *et al*, 2011), *DDX41* (Berger *et al*, 2017) and *GATA2* (Galera *et al*, 2018). Consideration of the time-

sensitivity whilst upholding a relative's right not to know would normally be addressed in genetic counselling around predictive testing.

Genes conferring germline/constitutional (heritable) susceptibility to haematological malignancies

DDX41

The *DDX41* gene encodes a DEAD-box RNA helicase (Polprasert *et al*, 2015). Germline pathogenic variants in *DDX41* are associated with an increased risk of haematological malignancy, mainly Myelodysplastic Syndrome (MDS) and Acute Myeloid Leukaemia (AML). The exact level of risk for *DDX41* carriers remains unclear. Cardoso *et al* (2016) compared the frequency of loss of function variants in MDS and secondary AML with the frequency seen in The Exome Aggregation Consortium (ExAC) database (Karczewski *et al*, 2017). Allowing for a background risk of developing MDS/AML as 1/100, they suggested a relative risk for *DDX41* carriers of 7.51. Studies in cohorts of MDS/AML patients with a germline *DDX41* pathogenic variant have found 27-43% have a family history of haematological malignancy or cytopaenias (Sébert *et al*, 2019; Bannon *et al*, 2020), yet *de novo* germline variants are rare and have not been reported to date (Churpek *et al*, 2021). Diagnosis of malignancy is most commonly in the sixth or seventh decade, showing overlap with sporadic AML, and there is a higher penetrance in men compared to women (Quesada *et al*, 2019; Sébert *et al*, 2019). Heterozygous carriers of a germline pathogenic variant in *DDX41* often have normal blood counts until a malignancy develops (Lewinsohn *et al*, 2016). However, a personal history of cytopaenia was reported in 22/33 *DDX41* carriers in the USA by Bannon *et al* (2020) and 15/33 *DDX41* carriers in France by Sébert *et al* (2020). No evidence-based guidelines for surveillance exist. Expert opinion has suggested Complete Blood Count (CBC) and clinical examination every 6-12 months, with recognition of a current lack of prospective data to inform this (Godley & Shimamura, 2017; Baliakas *et al*, 2019).

CEBPA

In 2004, the first family with a germline pathogenic variant in *CEBPA* was reported, including three relatives with AML (Smith et al, 2004). In 2015, analysis of 10 further families with pathogenic *CEBPA* variants was reported, including 24 individuals with AML (Tawana *et al*, 2015). Penetrance was 80% for development of AML in confirmed or obligate carriers. Median age of AML onset was 24.5 years (range, 1.75-46 years). Relapse risk was high (56% over 10 years) and characterised by an absence of *CEBPA* variants found in the diagnostic samples, with presence of different *CEBPA* variants in relapse samples, likely representing new leukaemic clones. From the limited number of families with a germline pathogenic variant, penetrance of AML appears to be high with onset at a younger age than sporadic AML. Most germline pathogenic variants have been identified in the N-terminal region of the gene. There is evidence to suggest that *CEBPA* pathogenic variants outside of the N-terminal region have lower penetrance of around 50% (Pathak *et al* 2016; Mendosa *et al* 2021). Heterozygous carriers of a germline pathogenic variant in *CEBPA* usually have normal blood counts until a malignancy develops (Tawana *et al*, 2017). Expert opinion has suggested CBC every 6-12 months, with bone marrow evaluation after CBC abnormalities (Tawana *et al*, 2010; Godley & Shimamura, 2017).

RUNX1

Germline pathogenic variants in *RUNX1* have been established as a cause of familial platelet disorder and predisposition to haematological malignancy since the late 1990s (Song *et al*, 1999). Over 130 families with *RUNX1*-Familial Platelet Disorder and Associated Myeloid Malignancies (*RUNX1*-FPDMM) have been described, with a wide spectrum of variants and no obvious correlation of variant sub-type with penetrance or expressivity (Brown *et al*, 2020; DiFilippo *et al*, 2020). Median age of onset of haematological malignancy is 33 years, with a range from early infancy to later adulthood (Brown *et al*, 2020). Symptoms present as easy bleeding/bruising (~90%), MDS/AML (~25-50%) and eczema (up to 50%), with only a minority of carriers remaining symptom-free (Brown *et al*, 2020). Expert opinion guidelines recommend CBC every 3-4 month, clinical examination annually and bone marrow evaluation if either are abnormal, with dermatology review as needed (Deuitch *et al*, 2021).

ANKRD26

In 2011, six different pathogenic variants in *ANKRD26* were described in nine families with autosomal dominant thrombocytopaenia (Pippucci *et al,* 2011). Subsequent analysis of 21 families with *ANKRD26* pathogenic variants showed that thrombocytopaenia and bleeding tendency is usually mild (Noris *et al*, 2011). In 2013, a new series of 118 individuals who were confirmed or very likely *ANKRD26* carriers showed that 10 of these individuals had developed myeloid malignancy (Noris *et al*, 2013). Combining this series with 108 *ANKRD26* carriers already reported showed 4.9% had acute leukaemias, 2.2% had MDS and 1.3% had chronic myeloid leukaemia (CML). No evidence-based guidelines for surveillance exist. Expert opinion has suggested annual Complete Blood Count (CBC) with bone marrow evaluation if CBC is abnormal (Perez Botero *et al*, 2018).

ETV6

In 2015, three unrelated families were reported with different germline pathogenic variants in *ETV6*, demonstrating segregation with thrombocytopaenia and haematological malignancy (Zhang *et al,* 2015). Subsequently, two unrelated families with *ETV6* germline pathogenic variants segregating with thrombocytopaenia and ALL were described after screening 588 paediatric leukaemia cases in a paediatric cancer database (Topka *et al*, 2015). No *ETV6* germline pathogenic variants were identified in children with other types of cancer in this database. In a study of 4405 children with ALL, *ETV6* germline analysis revealed 31 variants in 35 patients (0.79%), with 15/31 (48%) predicted to be deleterious variants (Moriyama *et al*, 2015). Children with an *ETV6* germline variant and ALL were on average older than children with ALL and no *ETV6* variants: 10.2 years (IQR 5.3-13.8) versus 4.7 years (IQR 3.0-8.7). Evidence so far has established the association of *ETV6* with mild to moderate thrombocytopaenia and a predisposition to acute leukaemias, whilst the degree of risk elevation remains unclear. No evidence-based guidelines for surveillance exist.

GATA2

A range of malignant and non-malignant phenotypes has been described in individuals and families with inherited heterozygous pathogenic variants in *GATA2*. Since 2011, there are more than 100 families and more than 122 carrier individuals reported with an associated phenotype (Brown *et al,* 2020). *GATA2*-deficiency syndrome is associated with disorders of haematopoiesis, lymphatics and immunity in children and adults, with overall penetrance estimated at over 90% in some families

(Collin *et al,* 2015). The risk of developing myeloid malignancy is estimated to be around 75%, although this is likely to include ascertainment bias, with median age of onset in the second decade (Wlodarski *et al*, 2017). *GATA2* carrier families may be more easily identified clinically compared to other predisposition genes due to their syndromic features, although presentation is variable. No evidence-based surveillance exists, but suggested published guidelines advise serial CBC and annual bone marrow examination, with involvement of other medical specialties as needed (Collin *et al,* 2015).

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