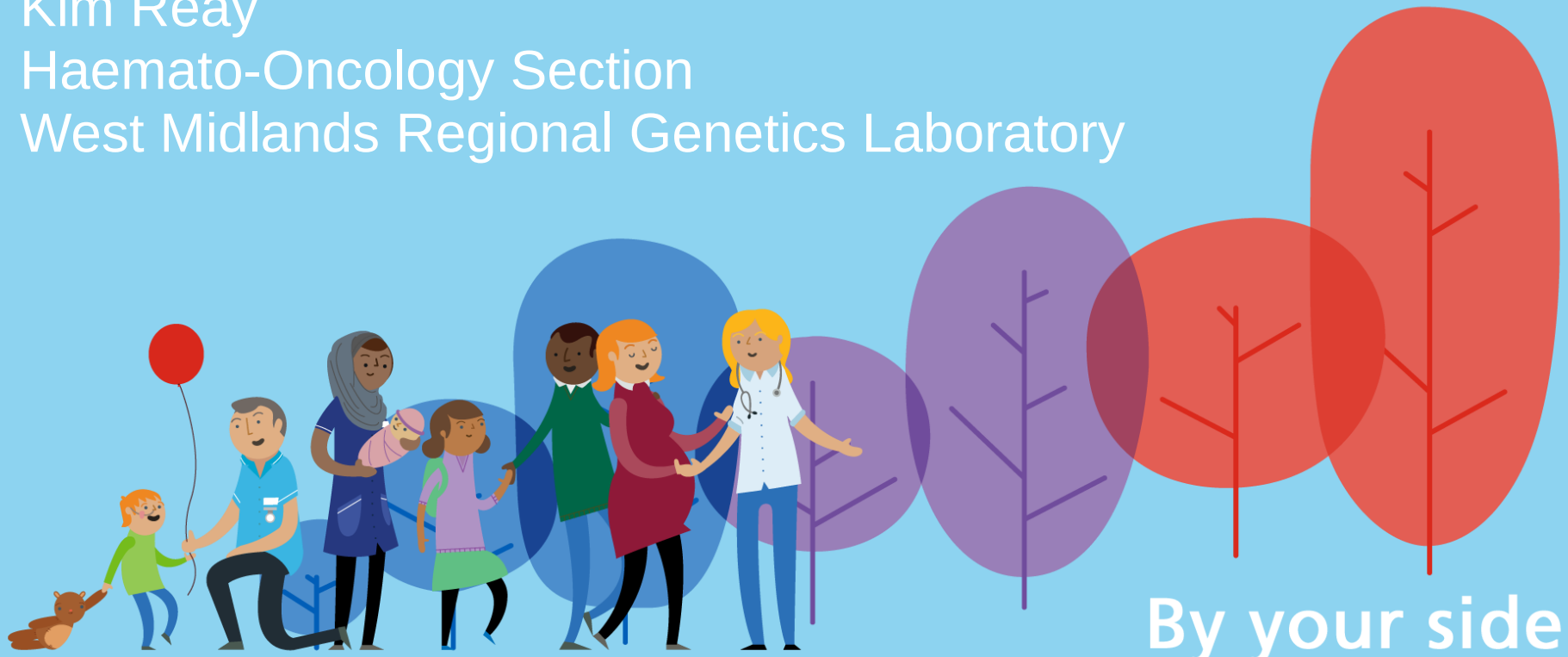
NHS
Birmingham Women's and Children's
NHS Foundation Trust

Identifying Potential Germline Variants During Somatic Testing: a Selection of Challenging Cases

Kim Reay
Haemato-Oncology Section
West Midlands Regional Genetics Laboratory



Overview

NHS
Birmingham Women's and Children's
NHS Foundation Trust

- Case 1: *TP53* variant in MDS patient
  - on-tumour potential germline finding
- Case 2: *KRAS* variant in JMML patient
  - choice of tissue for confirmation
- Case 3: *RUNX1* VUS in MDS patient
  - predictive testing of potential sibling donors pre-SCT

Case 1: Clinical details

Birmingham Women's and Children's NHS Foundation Trust

- 57yr old male with high risk MDS
  - ?aplastic anaemia, fatigue, macrocytic anaemia
- SNP array: abnormal clone with del(20q)

- Myeloid NGS panel requested

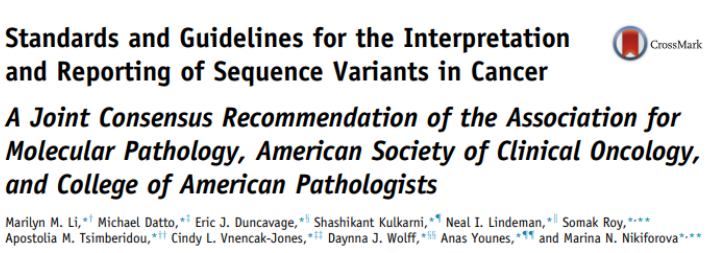
Case 1: NGS results and somatic variant interpretation

NHS
Birmingham Women's and Children's
NHS Foundation Trust

- Analysed in silico panel of 24 genes
  - Nextera version of Illumina TruSight Myeloid Sequencing panel

***TP53* c.542G>A p.(Arg181His) 53% VAF**	
In-house data	Previous report by Inherited Cancer team
Population databases	Absent/low level
Somatic databases	Recurrent in COSMIC and IARC
ClinVar	Pathogenic/Likely pathogenic. Reported in Li-Fraumeni syndrome/LFS (not classic)
Functional data	Defective/reduced promoter binding activity in DNA-binding domain
In silico tools	Damaging
Classification	**Clinically significant**

***TP53* c.298C>T p.(Gln100*) 6% VAF**	
In-house data	Novel
Population databases	Absent/low level
Somatic databases	Recurrent in COSMIC
ClinVar	Pathogenic/Likely pathogenic. Reported in Li-Fraumeni syndrome (not classic)
Functional data	DNA-binding domain
Classification	**Clinically significant**



SPECIAL ARTICLE

Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer

A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists

Marilyn M. Li, Michael Datto, Eric J. Duncavage, Shashikant Kulkarni, Neal I. Lindeman, Somak Roy, Apostolia M. Tsimberidou, Cindy L. Vnencak-Jones, Daynna J. Wolff, Anas Younes, and Marina N. Nikiforova

Case 1: NGS report

Birmingham Women's and Children's NHS Foundation Trust

- Deletions of 20q and *TP53* variants both recurrent in MDS
  - del(20q) classified in the good cytogenetic risk group (IPSS-R)
  - TP53 variants usually associated with -5/5q-, 7q-, complex karyotypes (adverse prognosis)
- Presence of del(20q) & *TP53* variants represents unexpected finding
  - Prognosis uncertain
- The *TP53* c.542G>A p.(Arg181His) variant with a VAF of 53% may be somatic or germline in origin

Case 1: Germline variant interpretation

Birmingham Women's and Children's NHS Foundation Trust

- Seen previously by Solid Cancer Team
  - Not associated with classic Li-Fraumeni syndrome
    - Later onset

TP53 c.542G>A p.(Arg181His) 53% VAF	
PS3 Moderate	Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product
PM1	Located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation
PM5	Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before
PP3	Multiple lines of computational evidence support a deleterious effect on the gene or gene product
Classification	**Likely pathogenic**

Case 1: NGS report

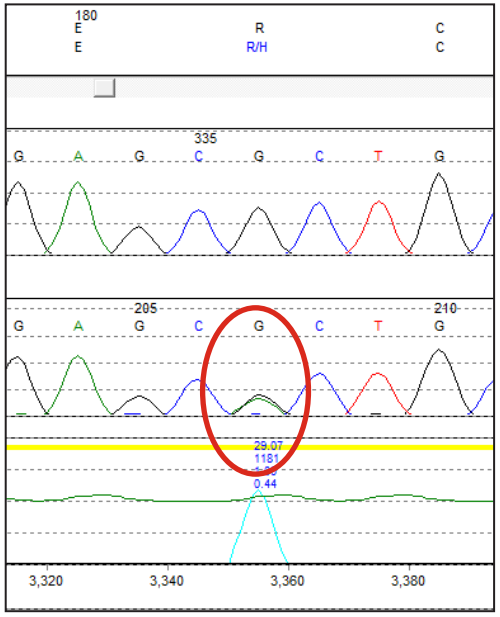
Birmingham Women's and Children's NHS Foundation Trust

- Deletions of 20q and *TP53* variants both recurrent in MDS
  - del(20q) classified in the good cytogenetic risk group (IPSS-R)
  - TP53 variants usually associated with -5/5q-, 7q-, complex karyotypes (adverse prognosis)
- Presence of del(20q) & *TP53* variant represents unexpected finding
  - Prognosis uncertain
- The *TP53* c.542G>A p.(Arg181His) variant with a VAF of 53% may be somatic or germline in origin
- This variant reported in both somatic cancer and hereditary LFS (reduced penetrance)
- Recommend patient referred to Clinical Genetics

Case 1: Follow-up

Birmingham Women's and Children's NHS Foundation Trust

- Result rang out to Consultant Haematologist
  - Germline testing requested ASAP
  - Long waiting time for patients to obtain CG appt
- Arranged meeting with Consultant Clinical Geneticist
  - Haematology request appropriate if result alters clinical management
- Extracted DNA from non-myeloid cells (CD3+ T-cells)
  - Heterozygous for TP53 variant
    - Germline variant



Case 1: Germline report

Birmingham Women's and Children's NHS Foundation Trust

- Result supports a diagnosis of LFS dependent on clinical criteria and family history
- Recommend patient and family referred to Clinical Genetics
- Presymptomatic testing available to at risk relatives if referred via Clinical Genetics

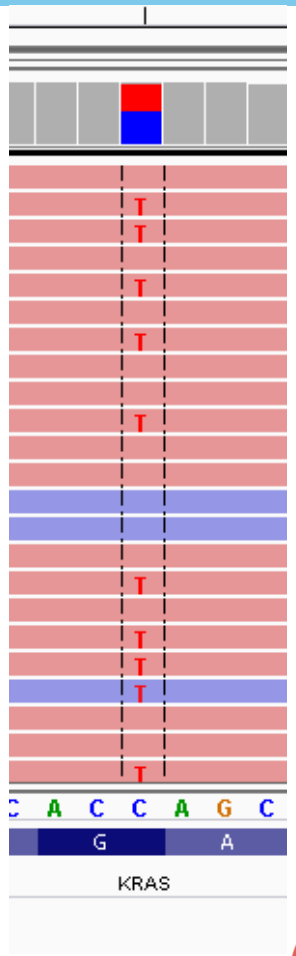
Key learning points:

- Need for considered/multi-disciplinary approach to dealing with potential pertinent germline findings
- LFS phenotype may change
  - Leukaemia rarely reported as 1st cancer in LFS in literature

Case 2: Clinical details

Birmingham Women's and Children's NHS Foundation Trust

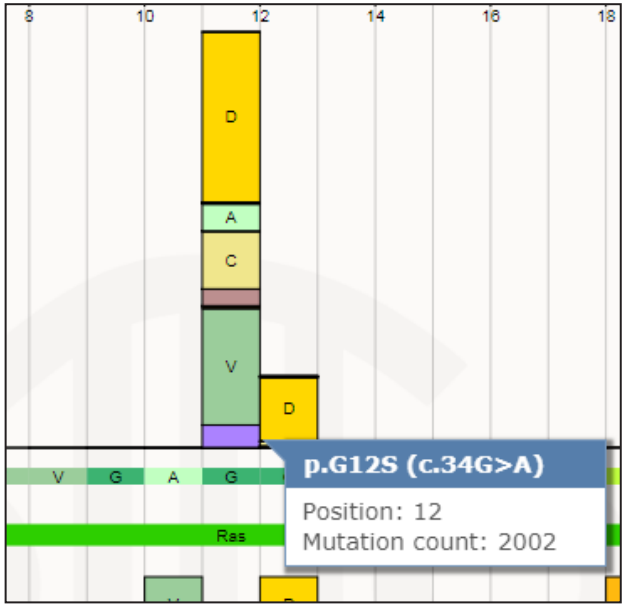
- 4 month old boy with ?JMML or metabolic disorder
  - Hepatosplenomegaly, FTT
- Germline
  - Array: no clinically significant copy number imbalance
- Somatic
  - G-band analysis: ANK
  - FISH: no evidence monosomy 7
  - NGS analysis using 32 gene JMML panel
    - Missense variant c.34G>A p.(Gly12Ser) in *KRAS* with 47% VAF

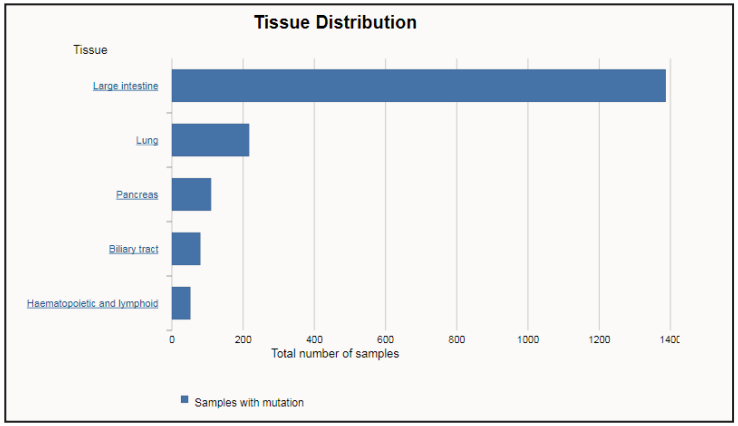


Case 2: Somatic variant classification

Birmingham Women's and Children's NHS Foundation Trust

***KRAS* c.34G>A p.(Gly12Ser) 47% VAF**	
In-house data	Not observed
Population databases	Absent
Somatic databases	Highly recurrent in COSMIC, incl. 52 times in H&L tissue
ClinVar	Reported multiple times in a variety of tumour types including JMML and considered pathogenic in the majority of cases
In silico tools	Damaging
Classification	**Clinically significant**





Case 2: Germline variant classification

NHS
Birmingham Women's and Children's
NHS Foundation Trust

***KRAS* c.34G>A p.(Gly12Ser) 47% VAF**	
PP3	Multiple lines of computational evidence support a deleterious effect on the gene or gene product
PM1	Located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation
PM2	Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium
PS3	Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product
PS4_supp	PS4 – (Strong) The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls
Classification	**Pathogenic**

www.nature.com/ejhg

ARTICLE

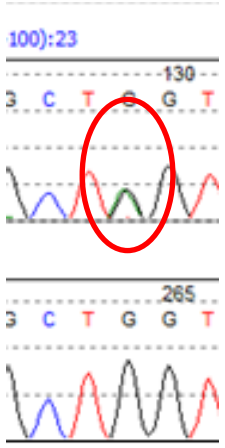
The lymphatic phenotype in Noonan and Cardiofaciocutaneous syndrome

Sarah Joyce[1], Kristiana Gordon[2], Glen Brice[1], Pia Ostergaard[3], Rani Nagaraja[4], John Short[1], Sandra Moore[1], Peter Mortimer[3] and Sahar Mansour*[,1,3]

Case 2: Report and follow-up

Birmingham Women's and Children's NHS Foundation Trust

- *KRAS* variants recurrent in myeloid neoplasia, including JMML
- In germline setting also associated with RASopathies (e.g.CFC syndrome)
  - Haematologist had no reason to suspect such a diagnosis

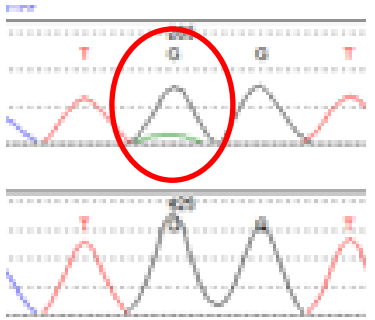


- Extracted DNA from non-myeloid cells (CD3+ T-cells)
  - Heterozygous for *KRAS* variant c.34G>A p.(Gly12Ser)
  - Likely to represent a germline variant, but somatic origin not completely excluded
  - Recommend analysis of non-haematopoietic tissue e.g. skin punch biopsy
  - Strongly recommended referral to Clinical Genetics

Case 2: Further follow-up

Birmingham Women's and Children's NHS Foundation Trust

- Treatment options:
  - JMML associated with RASopathy: Watch and wait
  - Acquired neoplastic origin: SCT



- DNA directly extracted from skin puncture
  - G12S variant at ~5%
    - ? contamination of the tissue with blood containing the acquired *KRAS* variant
    - ? somatic mosaicism

- Subsequently discussed at Genomics Tumour Advisory Board (GTAB)
  - Clinical Genetics asked to see the patient to perform a full assessment
    - Clinically fitting with mosaic Noonan syndrome
    - Characteristic facial appearance, short, mosaic skin pigmentation

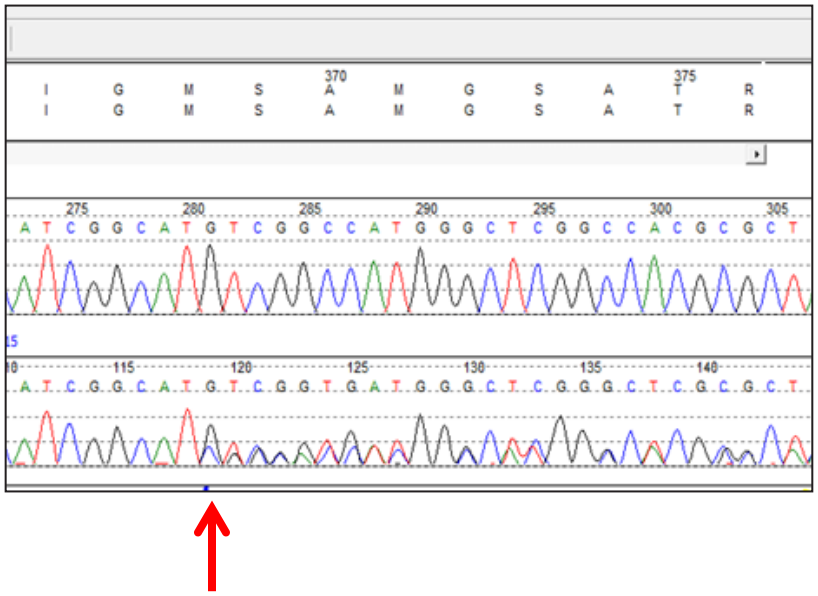
Key learning points:

- Use DNA from cultured fibroblasts as source of germline material in JMML
- Essential to stress the importance of a Clinical Genetics referral in view of immediate impact on patient management

Case 3: Clinical details

Birmingham Women's and Children's NHS Foundation Trust

- 52yr old male with high risk MDS
- Somatic testing in Glasgow identified *RUNX1* VUS
  - c.1098_1103dup p.(Ile366_Gly367)
- Patient being worked up for SCT
  - limited sibling donor options
- Variant identified in buccal scrape and skin punch biopsy
  - Confirmed VUS germline in origin



Case 3: Follow-up

NHS
Birmingham Women's and Children's
NHS Foundation Trust

- Aware of HLA-matched sibling donor
  - Offered Sanger sequencing analysis of the sibling
  - In view of the uncertain significance of the variant also recommended referral to Clinical Genetics
- Consultant Haematologist contacted HLA-matched potential sibling donor 1 (67yrs age):

Email from Consultant to sibling donor 1

- If you carry the same *RUNX1* mutation, then this would mean that you would not be a suitable donor.
- Hopefully it is unlikely that you will carry this mutation, since you are in good health and you have never had any blood problems.
- However, we would ideally like to check your blood cells before the transplant, to check that you do not have the same *RUNX1* mutation.

Case 3: Sibling donor 1

Birmingham Women's and Children's NHS Foundation Trust

- Heterozygous for c.1098_1103dup p.(Ile366_Gly367dup) in RUNX1
- Cannot determine whether this is the causative variant in the index patient
- F-MDS demonstrates incomplete penetrance
- Strongly recommend family referred to Clinical Genetics for counselling and segregation studies
- Outcome
  - Normal FBC
  - Arranging platelet aggregation studies
  - Family to have genetic counselling

Case 3: Sibling donor 2

Birmingham Women's and Children's NHS Foundation Trust

- 2nd potential sibling donor contacted for consent to be tested for *RUNX1* variant
  - Haploidentical HLA match

Email from Haematology consultant to sibling donor 2

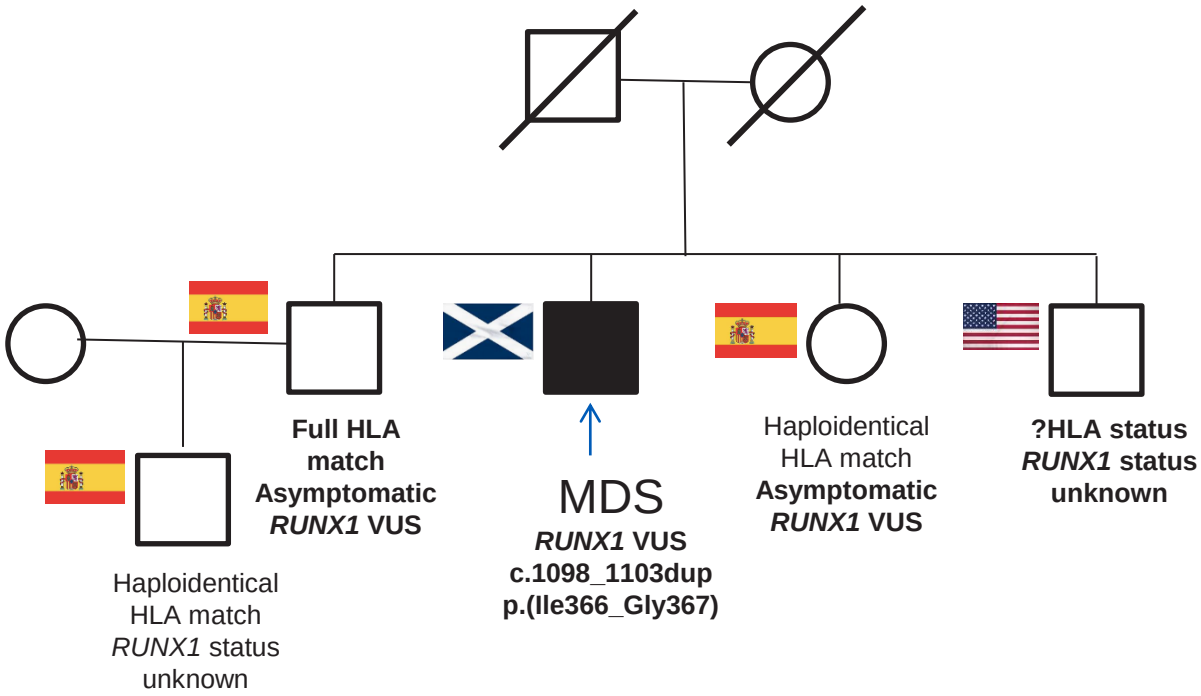
- Our best estimate at the moment is that it could be harmful, but that the increased risk of myelodysplasia and leukaemia is probably not that high.
- Told 50% risk carrying variant.
- Asked for consent to genetic testing but recommended genetic counselling before a decision is made.
- Will only consider use as PBSC donor if shown to be negative for *RUNX1*

- Also carry the *RUNX1* VUS ☹

Case 3: Pedigree

Birmingham Women's and Children's NHS Foundation Trust

- Family in different geographic locations



Key learning outcome

- Sometimes it is necessary to offer 'presymptomatic' testing for VUS

Summary

Birmingham Women's and Children's NHS Foundation Trust

- SVI ACGS guidelines being drafted and will include a section on the management of potential germline variants identified during somatic testing
- Example cases show not always easy to follow guidelines (exceptions to the rule)
- Effective management requires MDT approach: Clinical Scientist expertise in Haemato-Oncology and relevant germline disorders; Haematologists; Clinical Geneticists
- Well-established phenotypes i.e. LFS, may change
- JMML: T-cells not reliable source of material for germline confirmation

Acknowledgements

- Paula Page
- Jane Bryon
- Joanne Mason
- Mike Griffiths
- AML Team, WMRGL
- Solid Cancer Team, WMRGL
- Rare Disease Team, WMRGL
- Kenny Douglas, Beatson West of Scotland Cancer Centre
- Dr Jayashree Motwani, BWCH
- Dr Manoj Raghavan, UHB
- Clinical Genetics, BWCH
- Dr Sahar Mansour and Yasmin Clinch, St George's

Questions?

NHS
Birmingham Women's and Children's
NHS Foundation Trust