**Interpretive Educational Scheme (iED)**
**Clinical Scenario 2/2025 – Haematopoietic Stem Cell Transplant Case**

Dispatched on 26th August 2025

## Summary of Results

There were 41 responses received. 18 from laboratories based in the UK and Ireland (UK&I) and 23 from laboratories based in the rest of the World (RoW).

A 28-year-old female patient presented in January 2022. She had a prior diagnosis of common variable immunodeficiency (CVID), with whole-exome sequencing revealing a BACH2 mutation, and was later diagnosed with acute myeloid leukemia (AML). She began chemotherapy with the plan to proceed to a reduced-intensity allogeneic transplant from an unrelated donor.

Initial HLA typing was performed using peripheral blood-derived DNA, analysed by next-generation sequencing (NGS) at HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 loci. At the time of typing, the patient had 30% blasts. No relatives were available for HLA typing at that stage.

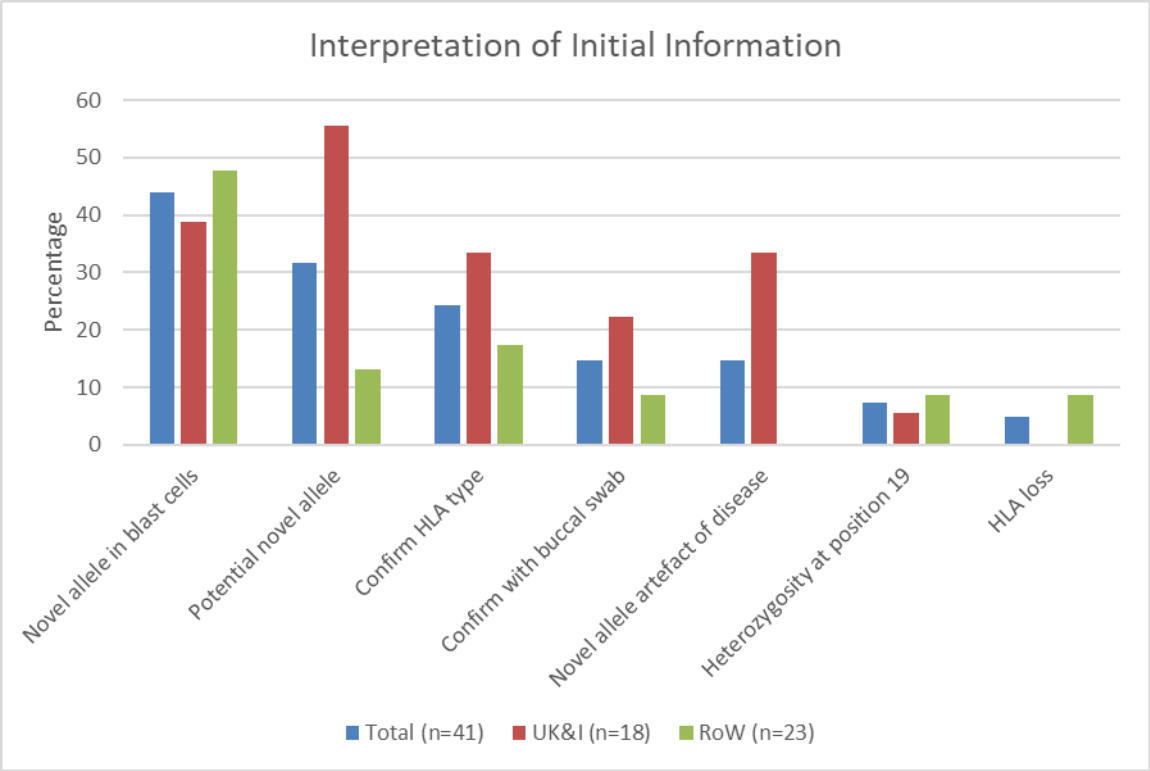
| | HLA-A | HLA-B | HLA-C | HLA-DRB1 | HLA-DQB1 | HLA-DPB1 |
|---|---|---|---|---|---|---|
| **Patient HLA Type** | *24:02<br>*31:01 | *44:02<br>*35:01 | *05:01<br>*04:01 | *01:01<br>*13:01 | *05:01<br>*06:03 | *02:01<br>*10:01 |

HLA typing by NGS resulted in uncertainty in the HLA type at the HLA-A locus. The patient was typed as HLA-A*24:02 with a query regarding whether the patient was HLA-A*31:01 wild type or HLA-A*31:01 novel allele, resulting in a null allele. The wild type A*31:01 allele has a C nucleotide at position 19, exon 1. The novel A*31:01 null allele has a T at this nucleotide position.

Peripheral blood sample HLA typed as HLA -A*24:02, *31:01 novel allele. 47% T nucleotides, 52% C, at nucleotide position 19 (exon 1).

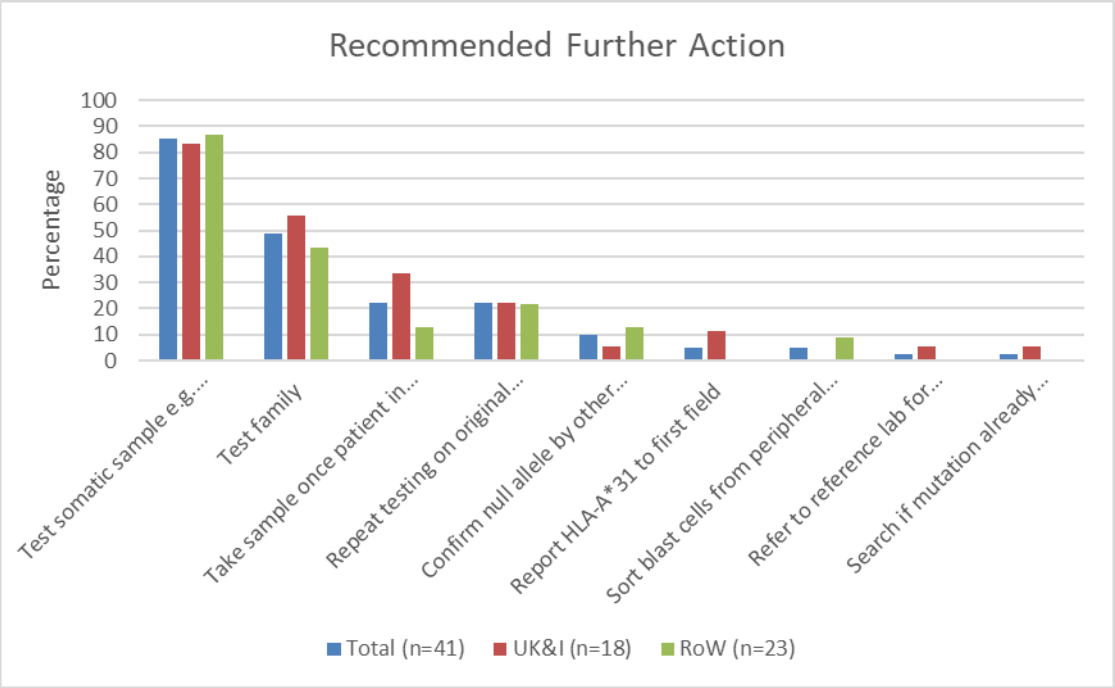
**Q1.1.** How would you interpret these results?

| Interpretation | Total (n=41) | | UK&I (n=18) | | RoW (n=23) | |
|---|---|---|---|---|---|---|
| | Count | % | Count | % | Count | % |
| Novel allele in blast cells | 18 | **44** | 7 | **39** | 11 | **48** |
| Potential novel allele | 13 | **32** | 10 | **56** | 3 | **13** |
| Confirm HLA type | 10 | **24** | 6 | **33** | 4 | **17** |
| Confirm with buccal swab | 6 | **15** | 4 | **22** | 2 | **9** |
| Novel allele artefact of disease | 6 | **15** | 6 | **33** | 0 | **0** |
| Heterozygosity at position 19 | 3 | **7** | 1 | **6** | 2 | **9** |
| HLA loss | 2 | **5** | 0 | **0** | 2 | **9** |




**Q1.2.** What further action (if any) would you take based on these results?

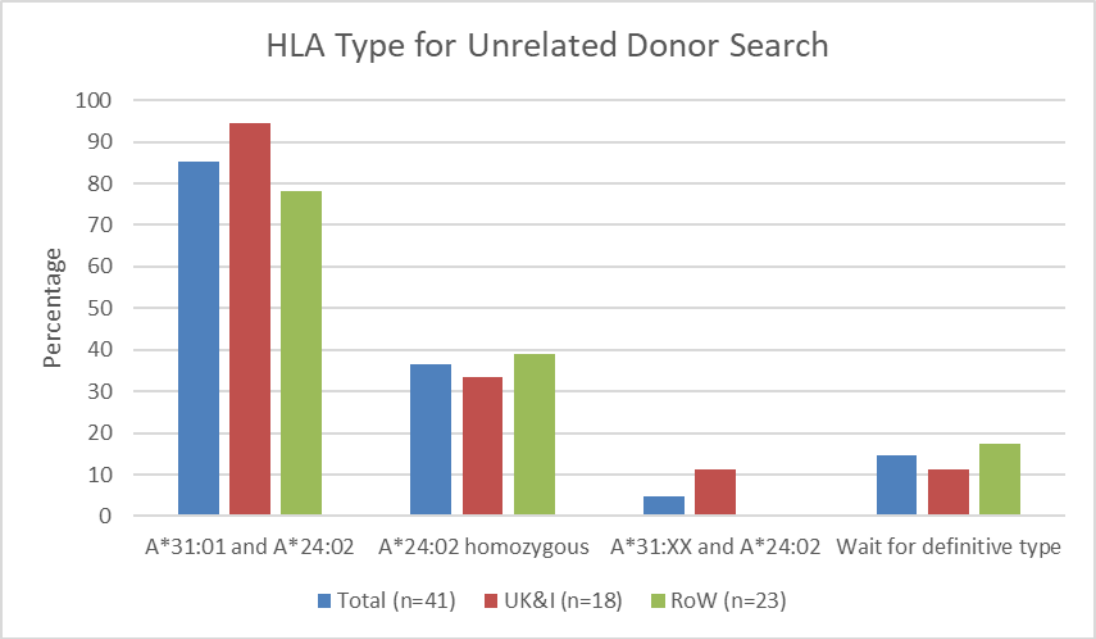
| Further Action | Total (n=41) | | UK&I (n=18) | | RoW (n=23) | |
|---|---|---|---|---|---|---|
| | Count | % | Count | % | Count | % |
| Test somatic sample e.g. buccal swab | 35 | **85** | 15 | **83** | 20 | **87** |
| Test family | 20 | **49** | 10 | **56** | 10 | **43** |
| Take sample once patient in remission | 9 | **22** | 6 | **33** | 3 | **13** |
| Repeat testing on original sample | 9 | **22** | 4 | **22** | 5 | **22** |
| Confirm null allele by other method e.g. serological typing | 4 | **10** | 1 | **6** | 3 | **13** |
| Report HLA-A*31 to first field | 2 | **5** | 2 | **11** | 0 | **0** |
| Sort blast cells from peripheral blood and retype | 2 | **5** | 0 | **0** | 2 | **9** |
| Refer to reference lab for sequencing | 1 | **2** | 1 | **6** | 0 | **0** |
| Search if mutation already published | 1 | **2** | 1 | **6** | 0 | **0** |




An urgent unrelated donor search was requested prior to any further testing.

**Q2.** Which HLA type would you use to initiate an unrelated search? Please give reasons for your response.

| Type for Unrelated Donor Search | Reasons | Total (n=41) | | UK&I (n=18) | | RoW (n=23) | |
|---|---|---|---|---|---|---|---|
| | | Count | % | Count | % | Count | % |
| A*31:01 and A*24:02 | | 35 | **85** | 17 | **94** | 18 | **78** |
| | More matches likely | 8 | **20** | 6 | **33** | 2 | **9** |
| | Match somatic cells | 5 | **12** | 2 | **11** | 3 | **13** |
| | No difference in antigen recognition site | 2 | **5** | 2 | **11** | 0 | **0** |
| | GvH risk lower | 2 | **5** | 1 | **6** | 1 | **4** |
| | Potential HvG | 2 | **5** | 0 | **0** | 2 | **9** |
| A*24:02 homozygous | | 15 | **37** | 6 | **33** | 9 | **39** |
| | If A*31 null allele | 2 | **5** | 1 | **6** | 1 | **4** |
| A*31:XX and A*24:02 | | 2 | **5** | 2 | **11** | 0 | **0** |
| | Initial search waiting for definitive type | 1 | **2** | 1 | **6** | 0 | **0** |
| Wait for definitive type | | 6 | **15** | 2 | **11** | 4 | **17** |

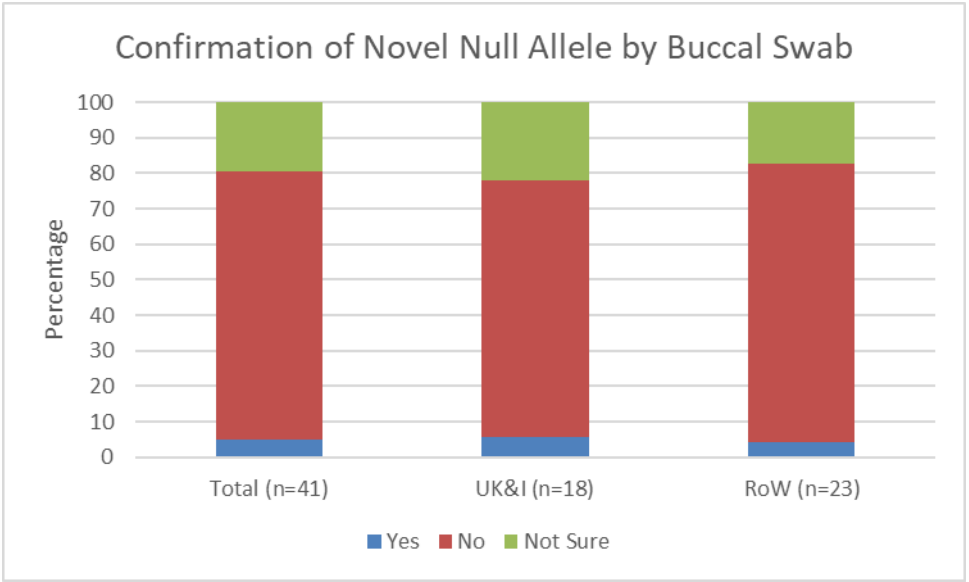



A buccal sample was subsequently obtained. This sample also showed the presence of a potential A*31:01 novel allele.

Buccal swab sample HLA typed as: HLA-A*24:02,*31:01 novel allele. 37% T nucleotides, 63% C, at nucleotide position 19 (exon 1).

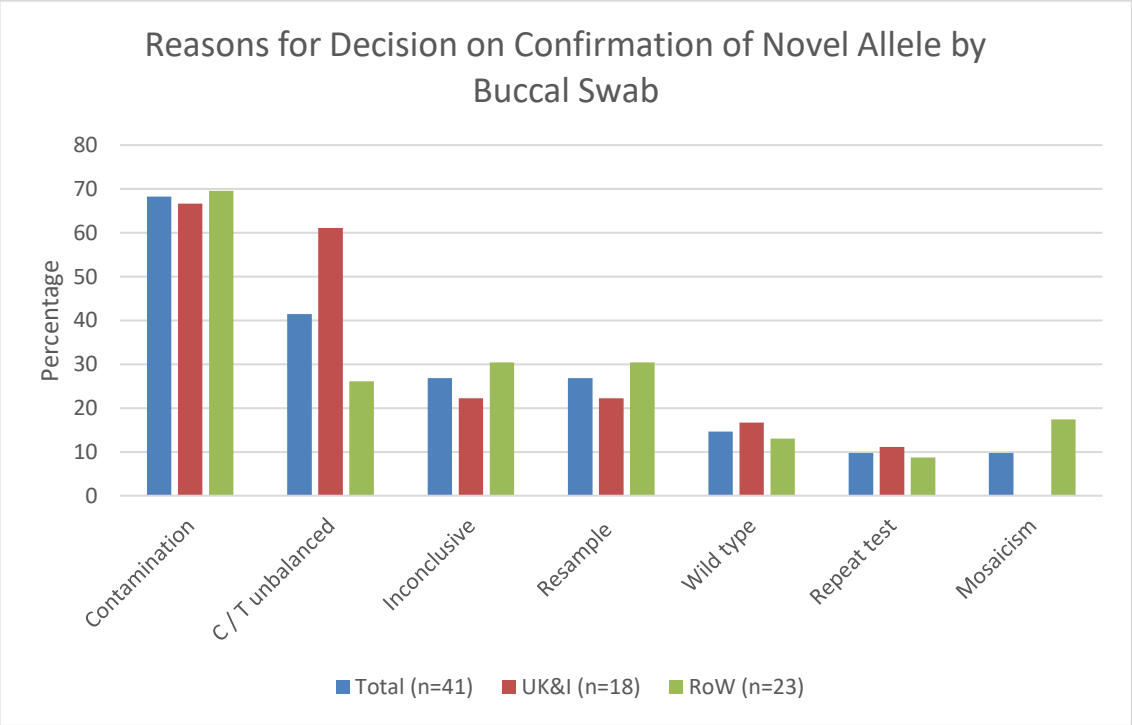
**Q3.1.** Would you consider the buccal swab derived HLA type as confirmation of novel null allele in this patient?

| Response | Total (n=41) | | UK&I (n=18) | | RoW (n=23) | |
|---|---|---|---|---|---|---|
| | Count | % | Count | % | Count | % |
| **Yes** | 2 | **5** | 1 | **6** | 1 | **4** |
| **No** | 31 | **76** | 13 | **72** | 18 | **78** |
| **Not Sure** | 8 | **20** | 4 | **22** | 4 | **17** |



**Q3.2.** Reasons for answer:

| Reasons | Total (n=41) | | UK&I (n=18) | | RoW (n=23) | |
|---|---|---|---|---|---|---|
| | Count | % | Count | % | Count | % |
| Contamination with other cell lines / DNA quality | 28 | **68** | 12 | **67** | 16 | **70** |
| C / T ratio unbalanced | 17 | **41** | 11 | **61** | 6 | **26** |
| Inconclusive - cannot exclude novel allele | 11 | **27** | 4 | **22** | 7 | **30** |
| Resample e.g. hair, skin | 11 | **27** | 4 | **22** | 7 | **30** |
| Buccal results suggest wild type | 6 | **15** | 3 | **17** | 3 | **13** |
| Test by another method | 4 | **10** | 2 | **11** | 2 | **9** |
| Mosaicism | 4 | **10** | 0 | **0** | 4 | **17** |

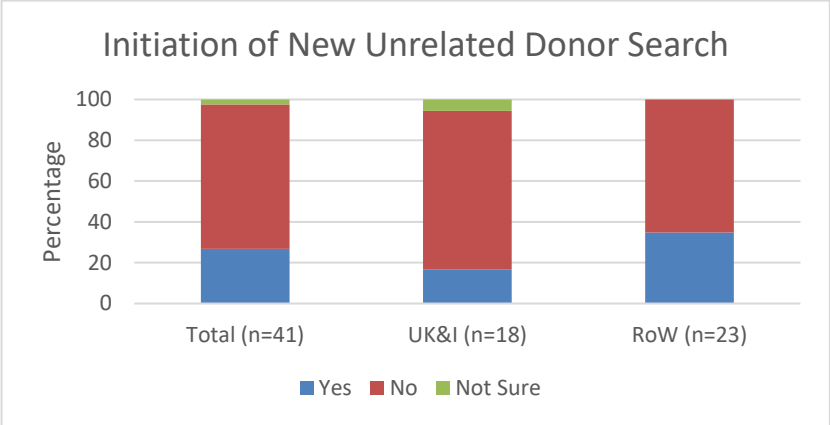



As no relatives were available for immediate confirmation of this novel allele, a skin biopsy sample was requested from the patient

Skin biopsy sample HLA typed as HLA-A*24:02, *31:01 wild type. 8% T nucleotides, 91% C, at position nucleotide position 19 (exon 1).

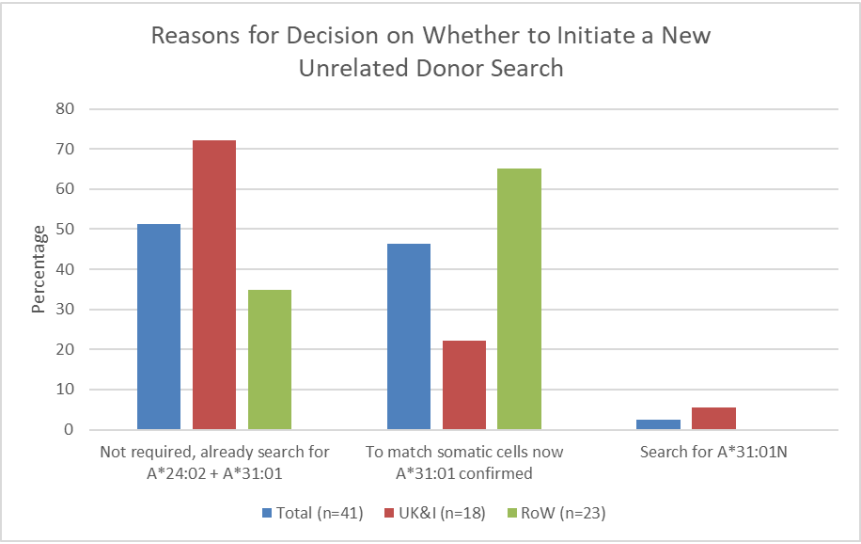
**Q4.1.** Would a new unrelated donor search be initiated following these results?

| Response | Total (n=41) | | UK&I (n=18) | | RoW (n=23) | |
|---|---|---|---|---|---|---|
| | Count | % | Count | % | Count | % |
| **Yes** | 11 | **27** | 3 | **17** | 8 | **35** |
| **No** | 29 | **71** | 14 | **78** | 15 | **65** |
| **Not Sure** | 1 | **2** | 1 | **6** | 0 | **0** |



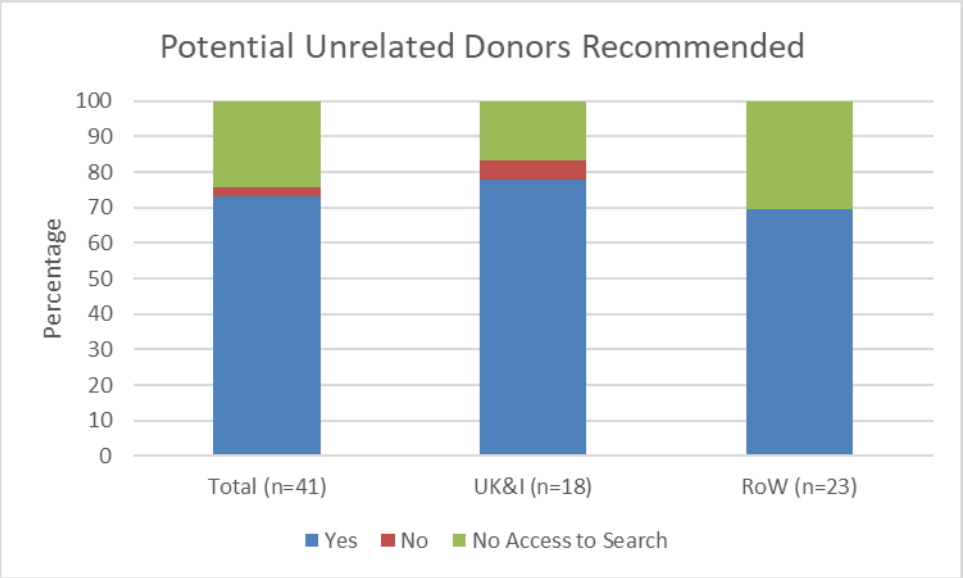

**Q4.2.** Reasons for answer:

| Reason for New Search | Total (n=41) | | UK&I (n=18) | | RoW (n=23) | |
|---|---|---|---|---|---|---|
| | Count | % | Count | % | Count | % |
| Not required, already search for A*24:02 + A*31:01 | 21 | **51** | 13 | **72** | 8 | **35** |
| To match somatic cells now A*31:01 confirmed | 19 | **46** | 4 | **22** | 15 | **65** |
| Search for A*31:01N | 1 | **2** | 1 | **6** | 0 | **0** |



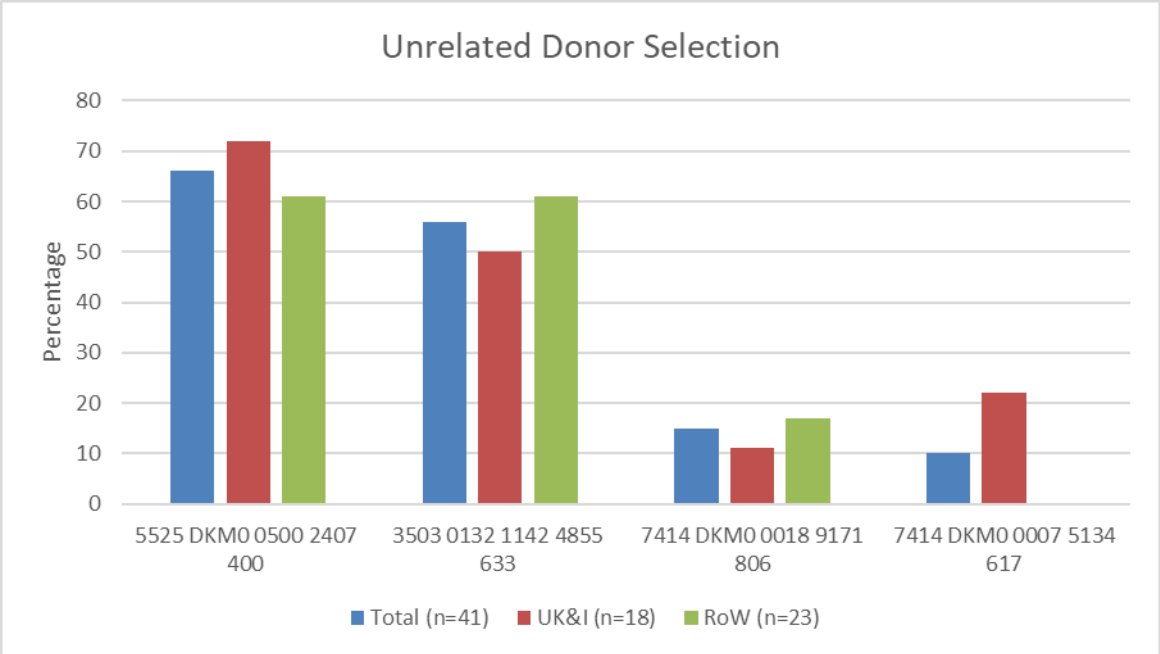
**Q5.1.** If you are able, run the patient on an unrelated donor search programme based on the patient type being HLA-A*24:02 *31:01. Are there any potential donor options that may be recommended for transplant?

| Response | Total (n=41) | | UK&I (n=18) | | RoW (n=23) | |
|---|---|---|---|---|---|---|
| | Count | % | Count | % | Count | % |
| Yes | 30 | **73** | 14 | **78** | 16 | **70** |
| No | 1 | **2** | 1 | **6** | 0 | **0** |
| No Access to Search | 10 | **24** | 3 | **17** | 7 | **30** |




**Q5.2.** If yes, provide the donor ID, and the reasons for selection of your **two** preferred donors

| Donor ID | Most Common Reasons Given for Donor Selection | | | | | | | | | | | Total (n=41) | | UK&I (n=18) | | RoW (n=23) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Age <30 years old | Male | Female | Accredited Registry | No DP Typing | Non-Permissive DP mismatch | ABO Group O | GvH Direction | 10/10 | 11/12 | 12/12 | Count | % | Count | % | Count | % |
| 5525 DKM0 0500 2407 400 | ✓ | ✓ | | ✓ | | ✓ | ✓ | ✓ | ✓ | ✓ | | 27 | **66** | 13 | **72** | 14 | **61** |
| 3503 0132 1142 4855 633 | ✓ | ✓ | | ✓ | ✓ | | ✓ | | ✓ | | ✓ | 23 | **56** | 9 | **50** | 14 | **61** |
| 7414 DKM0 0018 9171 806 | ✓ | | | ✓ | | ✓ | ✓ | ✓ | ✓ | | | 6 | **15** | 2 | **11** | 4 | **17** |
| 7414 DKM0 0007 5134 617 | ✓ | | ✓ | ✓ | | | | ✓ | ✓ | ✓ | | 4 | **10** | 4 | **22** | 0 | **0** |

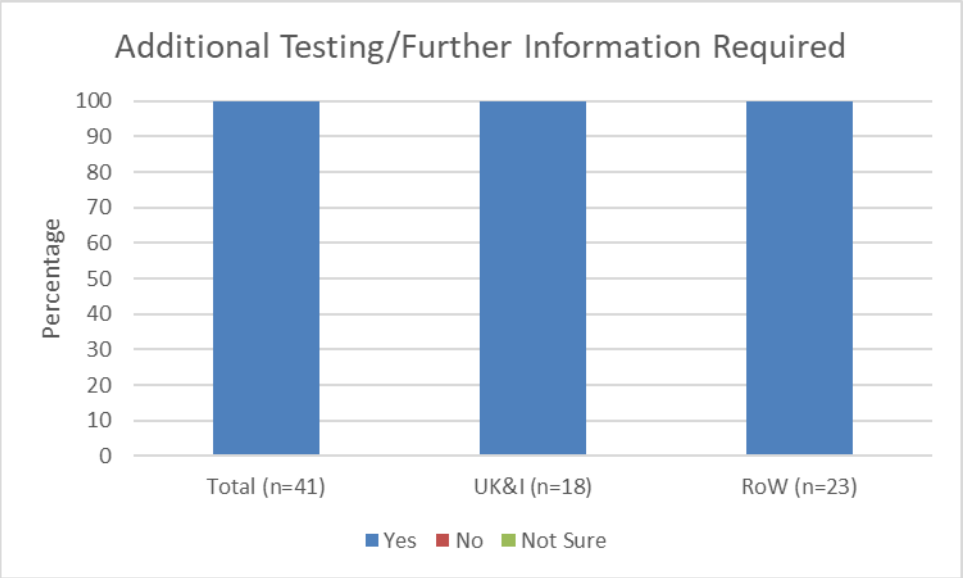



Due to clinical urgency, it was decided that her mother would be worked up as a haploidentical donor.

| | HLA-A | HLA-B | HLA-C | HLA-DRB1 | HLA-DQB1 | HLA-DPB1 |
|---|---|---|---|---|---|---|
| Patient HLA Type | *24:02 *31:01 | *44:02 *35:01 | *05:01 *04:01 | *01:01 *13:01 | *05:01 *06:03 | *02:01 *10:01 |
| Maternal HLA Type | *24:02 *03:01 | *44:02 *07:02 | *05:01 *07:02 | *01:01 *15:01 | *05:01 *06:02 | *02:01 *04:01 |

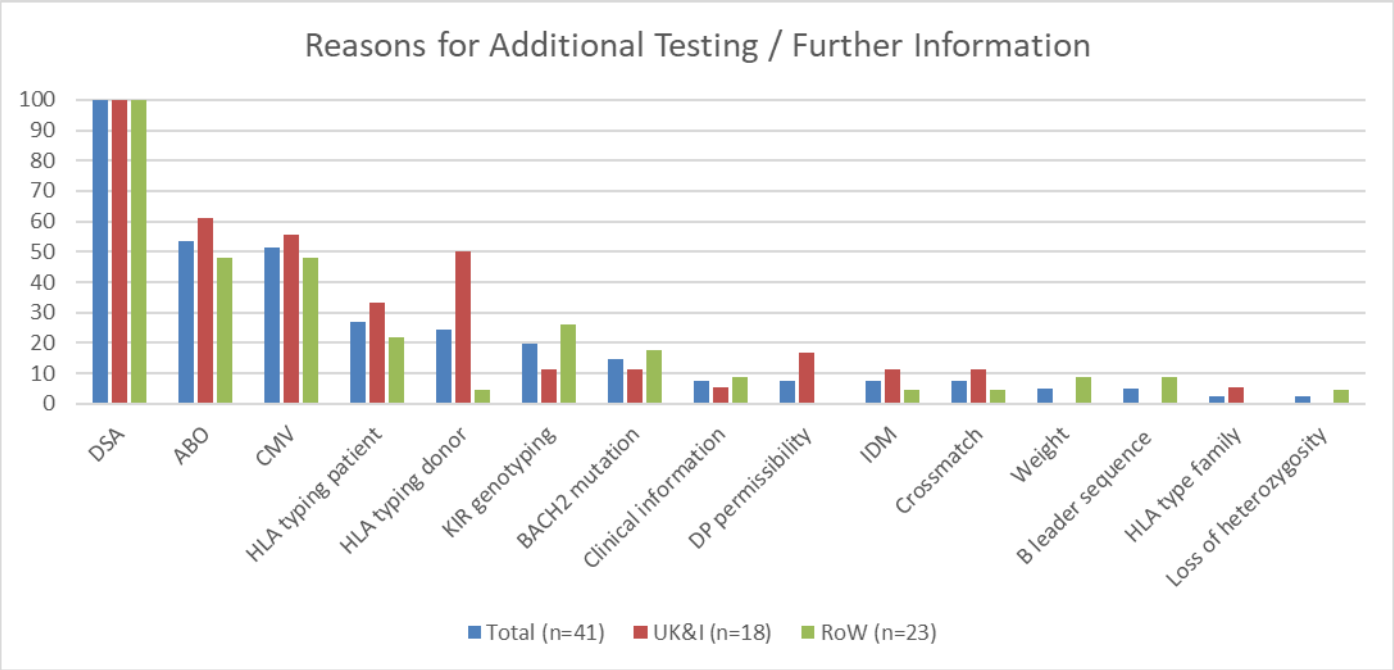
**Q6.1.** Would you perform additional testing or require any further information prior to selection of the related donor?

| Response | Total (n=41) | | UK&I (n=18) | | RoW (n=23) | |
|---|---|---|---|---|---|---|
| | Count | % | Count | % | Count | % |
| **Yes** | 41 | **100** | 18 | **100** | 23 | **100** |
| **No** | 0 | **0** | 0 | **0** | 0 | **0** |
| **Not Sure** | 0 | **0** | 0 | **0** | 0 | **0** |




**Q6.2.** Reasons for answer:

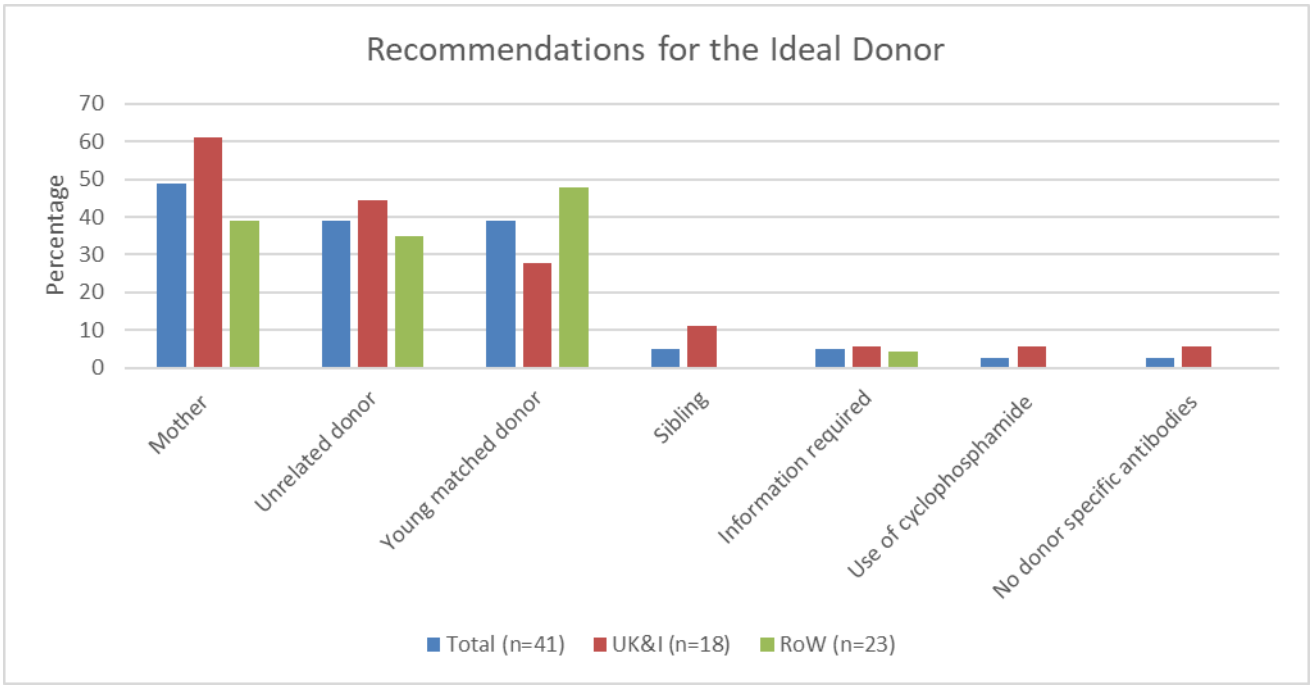
| Reasons for additional testing/further information | Total (n=41) | | UK&I (n=18) | | RoW (n=23) | |
|---|---|---|---|---|---|---|
| | Count | % | Count | % | Count | % |
| Donor specific antibodies | 41 | **100** | 18 | **100** | 23 | **100** |
| ABO testing | 22 | **54** | 11 | **61** | 11 | **48** |
| CMV testing | 21 | **51** | 10 | **56** | 11 | **48** |
| Extended/confirmatory HLA typing on patient | 11 | **27** | 6 | **33** | 5 | **22** |
| Extended/confirmatory HLA typing on donor | 10 | **24** | 9 | **50** | 1 | **4** |
| KIR genotyping | 8 | **20** | 2 | **11** | 6 | **26** |
| Genetic testing mother for BACH2 mutation | 6 | **15** | 2 | **11** | 4 | **17** |
| Clinical information on patient | 3 | **7** | 1 | **6** | 2 | **9** |
| DP permissibility assessment | 3 | **7** | 3 | **17** | 0 | **0** |
| Infectious disease marker testing on patient | 3 | **7** | 2 | **11** | 1 | **4** |
| Crossmatch | 3 | **7** | 2 | **11** | 1 | **4** |
| Patient and donor weight | 2 | **5** | 0 | **0** | 2 | **9** |
| B leader sequence assessment | 2 | **5** | 0 | **0** | 2 | **9** |
| HLA type family for back-up donors | 1 | **2** | 1 | **6** | 0 | **0** |
| Loss of heterozygosity assessment | 1 | **2** | 0 | **0** | 1 | **4** |




**The patient is negative for HLA antibodies.**

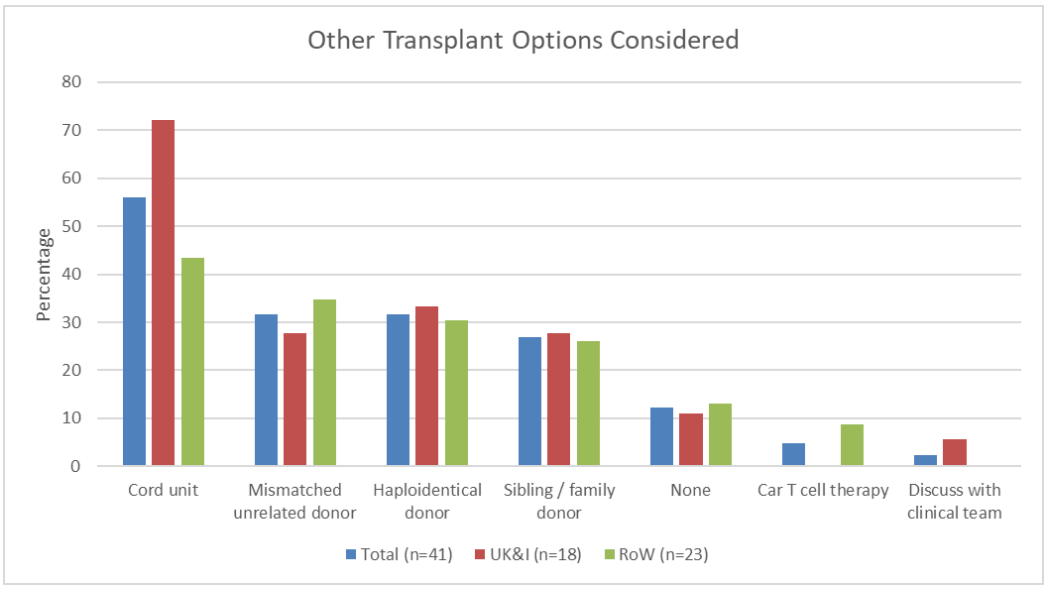
**Q7.** What recommendations (if any) would you give regarding the ideal donor for this patient?

| Recommendations | Total (n=41) | | UK&I (n=18) | | RoW (n=23) | |
|---|---|---|---|---|---|---|
| | Count | % | Count | % | Count | % |
| Mother preferred (faster work-up) | 20 | **49** | 11 | **61** | 9 | **39** |
| Unrelated donor (better match) | 16 | **39** | 8 | **44** | 8 | **35** |
| Young donor, 10/10 or 12/12 HLA match, CMV match | 16 | **39** | 5 | **28** | 11 | **48** |
| Fully HLA matched or haploidentical sibling | 2 | **5** | 2 | **11** | 0 | **0** |
| More information required / discuss with clinical colleagues | 2 | **5** | 1 | **6** | 1 | **4** |
| Use of cyclophosphamide increases risk of infection | 1 | **2** | 1 | **6** | 0 | **0** |
| No donor specific antibodies to impact on selection | 1 | **2** | 1 | **6** | 0 | **0** |



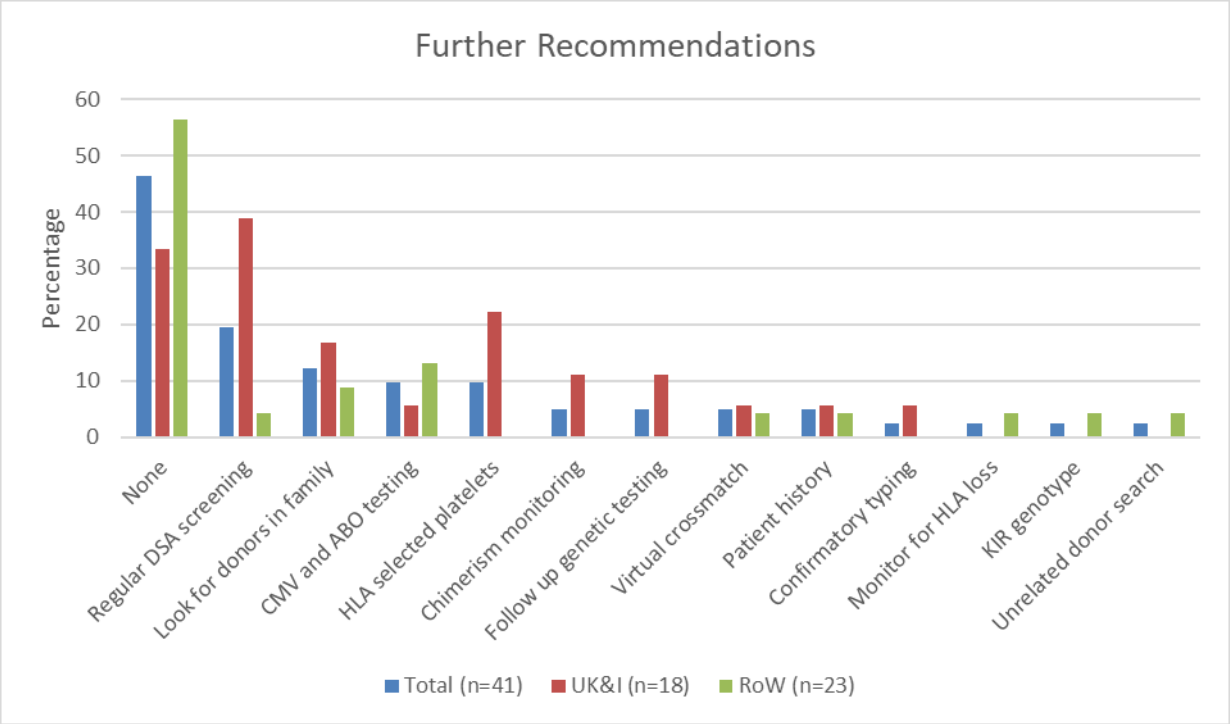

**Q8.1.** Would you consider any other transplant options?

| Other transplant options | Total (n=41) | | UK&I (n=18) | | RoW (n=23) | |
|---|---|---|---|---|---|---|
| | Count | % | Count | % | Count | % |
| Cord unit | 23 | **56** | 13 | **72** | 10 | **43** |
| Mismatched unrelated donor | 13 | **32** | 5 | **28** | 8 | **35** |
| Haploidentical donor | 13 | **32** | 6 | **33** | 7 | **30** |
| Sibling / family donor | 11 | **27** | 5 | **28** | 6 | **26** |
| None | 5 | **12** | 2 | **11** | 3 | **13** |
| Car T cell therapy | 2 | **5** | 0 | **0** | 2 | **9** |
| Discuss with clinical team | 1 | **2** | 1 | **6** | 0 | **0** |



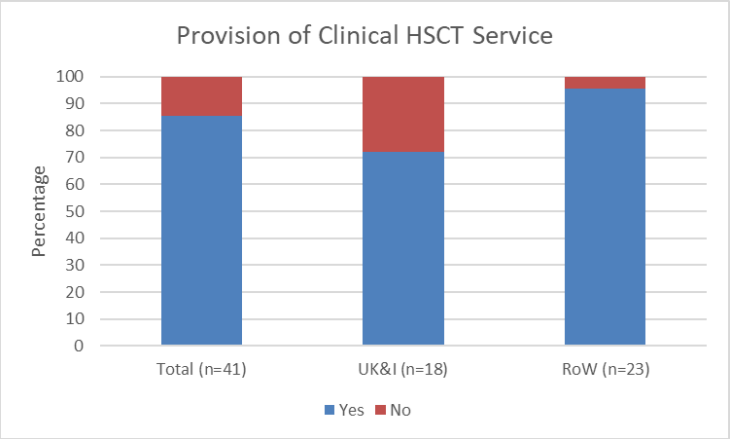
**Q8.2.** Any other recommendations?

| Final recommendations | Total (n=41) | | UK&I (n=18) | | RoW (n=23) | |
|---|---|---|---|---|---|---|
| | Count | % | Count | % | Count | % |
| None | 19 | **46** | 6 | **33** | 13 | **57** |
| Regular DSA screening | 8 | **20** | 7 | **39** | 1 | **4** |
| Look for donors in family | 5 | **12** | 3 | **17** | 2 | **9** |
| CMV and ABO testing | 4 | **10** | 1 | **6** | 3 | **13** |
| HLA selected platelets | 4 | **10** | 4 | **22** | 0 | **0** |
| Post-transplant chimerism monitoring | 2 | **5** | 2 | **11** | 0 | **0** |
| Follow up genetic testing (BACH2) | 2 | **5** | 2 | **11** | 0 | **0** |
| Virtual crossmatch | 2 | **5** | 1 | **6** | 1 | **4** |
| Patient history and weight | 2 | **5** | 1 | **6** | 1 | **4** |
| Confirmatory typing donor | 1 | **2** | 1 | **6** | 0 | **0** |
| Monitor for HLA loss | 1 | **2** | 0 | **0** | 1 | **4** |
| KIR genotype | 1 | **2** | 0 | **0** | 1 | **4** |
| International unrelated donor search | 1 | **2** | 0 | **0** | 1 | **4** |



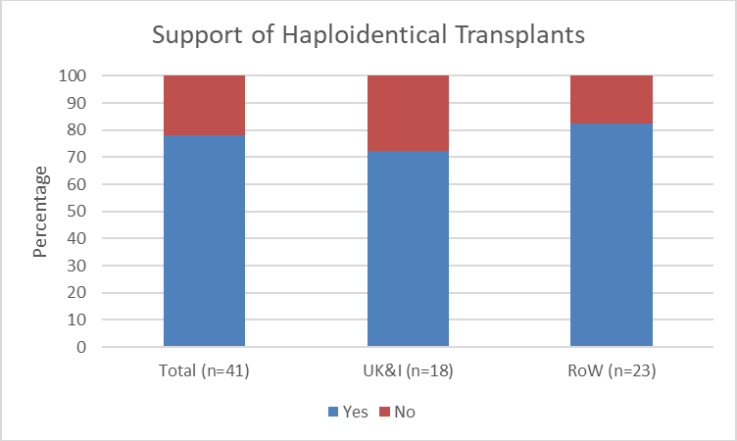

*Does your laboratory provide a clinical HSCT service?*

| Response | Total (n=41) | | UK&I (n=18) | | RoW (n=23) | |
|---|---|---|---|---|---|---|
| | Count | % | Count | % | Count | % |
| **Yes** | 35 | **85** | 13 | **72** | 22 | **96** |
| **No** | 6 | **15** | 5 | **28** | 1 | **4** |




*Does your laboratory routinely perform haploidentical transplants?*

| Response | Total (n=41) | | UK&I (n=18) | | RoW (n=23) | |
|---|---|---|---|---|---|---|
| | Count | % | Count | % | Count | % |
| **Yes** | 32 | **78** | 13 | **72** | 19 | **83** |
| **No** | 9 | **22** | 5 | **28** | 4 | **17** |




*Any other comments:*

- Should be checking if mutations are resulting in null alleles and how we utilise them for searches. Additionally skin biopsies may not be available for all labs so it is important to consider leukaemic infiltrate in samples.
- It would be useful to know the patient CMV status to help with donor selection. Read depth of NGS/how many copy of nucleotides present would be helpful to determine quality of test. Useful to know if blasts were in PB or BM.
- Would have also run A*24 homozygous search to review options in case of null allele confirmation.
- We perform NGS using One Lambda AllType™ FASTPLexTM NGS 11-Locus kits in combination with TypeStream™ Visual (TSV) 3.1 Analysis Software. High background is flagged as >20% at a particular position. We record all exonic high background positions for monitoring purposes.

- Due to the urgency of this case and the potential novel allele, senior staff would be consulted and further information sought from the clinical team regarding this patient's diagnoses.
- During the donor search would recommend calling donor 3503013211424855633 from Turkey as they are not DPB1 typed. If they are DPB1 permissive with the patient then they would be the preferred donor.
- More information required about the quality metrics of the NGS result e.g. expected nucleotide for A*24:02, read depth at the position in question/number of reads of each nucleotide, allele balance.
- There is a lot of information missing in this scenario that would be essential in donor selection.
- This case illustrates a likely hematopoietic-restricted A*31 variant; donor search should reflect confirmed germline typing to avoid unnecessary exclusions.
- Normally the MUD search results are already given in this educational scheme. In this way you prevent answers from labs not able to run the search themselves.
- In the real life we would have time to obtain a non blastic blood sample before searching for a donor, we would wait for 1st remission after induction, and ask for supplemental family typings to clarify the A*31:01 allele. This clinical test seems to us a little bit “artificial”, concerning the donor choice, because usually we have all the information at the beginning (patient CMV status and ABO group, family members).
- DNA Sequencing that is highly sensitive is important especially in detecting mutation in haematological cancer. The right tool helps in making the right decision.
- Collaboration with international registries widen unrelated donor pool and increase the chance to find a fully match donor.
- What will be the plans for chimerism and MRD monitoring?
- Mixed base signals occur when there are blast cells, where the signal for one HLA allele is stronger than another, can indicate the proportion of the patient's original cells versus mutated cells, and the complexity of HLA genes can make interpretation difficult.

***Comments and suggested responses from the UK H&I experts providing this scenario****

**Question 1**

The NGS results from peripheral blood require further investigation. There could be a number of issues impacting the result such as the presence of a novel allele, the result may be due to the patient's condition or there may be contamination present.

Recommended further action could include testing a somatic sample such as a buccal swab or skin sample, re-sampling once the patient is in remission, re-testing the original sample or testing by another method to confirm the presence of a null allele.

**Question 2**

The decision of which HLA type to use to perform a search for an unrelated donor would likely be shaped by clinical urgency. If there is time to wait for a definitive HLA type after further investigations have been performed then this is likely the preferred option. If urgency means the search needs to be initiated immediately it might be prudent to perform two searches using the wild type and another taking into account the impact of the novel null allele.

**Question 3**

The decision on whether the buccal swab confirms the novel null allele likely rests with local acceptance criteria. Due to the unbalanced ratios at position 19 it may be worth considering a further test using a skin sample to avoid contamination with disease-impacted cell lines.

**Question 4**

A new unrelated donor search may be required if the original search was not performed using the wild type HLA genotype i.e. A*31:01 and A*24:02.

**Question 5**

A number of unrelated donor options were available from international registries. The most popular donors selected were young, male donors from accredited registries with a minimum 10/10 HLA match.

**Question 6**

It may be worth considering whether additional testing such as screening for the presence of HLA donor specific antibodies if a mismatched donor is selected is required. Also, ABO type, CMV status and infectious disease marker testing could be requested.

**Question 7**

Recommendations for the ideal donor from the options available depend on the clinical urgency of the case. The patient's mother may offer a faster work-up than an unrelated donor. The unrelated donor options could, however, provide a better HLA match and younger donor.

**Question 8**

In discussion with the wider multi-disciplinary team options such as cord blood units could be considered.

***Please note:***

***These comments have been compiled by subject matter experts from the UK NEQAS for H&I Steering Committee in accordance with current guidelines. We accept that guidelines are not always explicit for every situation and therefore the responses may be aligned with the clinical practices of an individual transplant centre and may not be directly applicable across all settings. UK NEQAS are not necessarily endorsing these responses as the only correct action, just one possible view which, we acknowledge, may be biased towards UK practice.***