Haematological Malignancy Diagnostic Service

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Welcome to Spring

Spring is upon us technically speaking, although with the recent weather that's been quite hard to believe. We'd like to say a huge thank you to all the staff who struggled through the snow to get samples to us. There were very few cases where processing was delayed so please pass that on to local staff. It's been quite a while since our last edition so lots to catch up on and if there's anything else you feel it would be good to see in our next bulletin, please get in touch.

TP53 deletion or mutation? – that is the question

The *TP53* gene is located on chromosome 17 (17p13.1) and encodes the protein p53, which has many roles in defending the cell against DNA damage and, as a result, has an anticancer function. Dysfunction of the *TP53* gene/protein pathway is associated with an adverse prognosis in most cancers and in CLL has been associated with resistance to chemo-immunotherapy and a poor clinical outcome. Disruption of the *TP53* gene can occur by two main mechanisms; deletion and mutation, both of which are related to equally poor outcome. Disruption of both alleles of the gene are required to completely inactivate function, meaning many CLL patients may have both a deletion and a mutation of *TP53* gene.

Deletion of the 17p (*TP53*) chromosome region is demonstrated in 8-10% of CLL cases. HMDS currently uses FISH (fluorescent in situ hybridisation) on fixed whole cells to identify loss of the 17p region. This generally detects a mono-allelic loss of 17p (signal pattern 1Red and 2Green spots, where green is the control probe), indicating that one allele remains intact. Bi-allelic deletions are not seen.

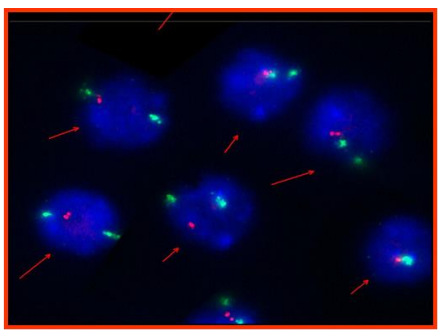
Mutations in *TP53* are seen in a smaller proportion of CLL cases (5%) at presentation, although frequency of mutations can increase at disease progression. Approximately 50% of these cases will have a mutation in the majority of cells but the remainder can have sub-clonal deletions which are below the sensitivity of routine approaches.

At present all CLL patients requiring treatment will have FISH performed to look for deletion of *TP53*. This is a relatively easy test and is very reliable. Mutational analysis is more challenging – the *TP53* gene is large, spanning 10 exons, with most mutations restricted to exons 5-9 of the gene. We currently use long distance PCR amplification of exons 5 to 9 of the *TP53* gene using genomic DNA followed by conventional Sanger sequencing. Four separate sequencing reactions are needed to cover the amplified region. Sanger sequencing can identify the majority of small scale mutations (nonsense, missense, small insertions or deletions) but these need to be distinguished from polymorphisms which do not alter the gene function and this introduces further complexity. The sensitivity of this current approach is also quite low, (approximately 15%). We are in the process of transitioning to using high-throughput sequencing for *TP53* mutational analysis, which will increase sensitivity to 5-10%, but this is an expensive approach. We are attempting to identify external funding for this but if that is not possible this will have to involve extra cost for the referrer.

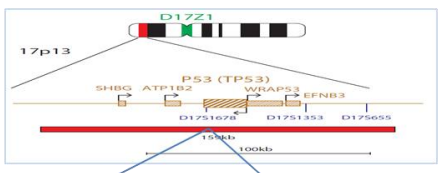
Judging by the request forms we see in HMDS for CLL patients, it appears some of our users are uncertain which test for *TP53* aberration is most applicable, with 'deletion' and 'mutation' being requested interchangeably.

Current advice would be to request FISH in the first instance for all pre-treatment patients. Please be sure you are requesting the appropriate test (TP53 deletion/FISH for deletion status and TP53 mutation/sequencing for TP53 mutations). If you have any questions regarding the most appropriate test please contact HMDS and speak to one of the senior Clinical Scientists.

a



b



Query 549 CAATGCCGCTGAGTATCTGCTGAGCAGCGGGATCAATGGCAGCTTCTTGGTGCGTGAGAG 608
Sbjct 766 CAATGCCGCTGAGTATCCGCTGAGCAGCGGGATCAATGGCAGCTTCTTGGTGCGTGAGAG 825

Figure a. FISH image showing TP53 deletion (one red spot) with 2 copies of chromsome 17 centromere (2 green spots).

Figure b. Probe map of Cytocell TP53 probe. FISH cannot detect mutations. DNA is extracted from the cells and long distance PCR carried out prior to Sanger sequencing. A skilled scientist needs to 'read' the sequence to identify the mutation, the example shown in red text shows '**TC**' base pairing identifying a possible mutation.

100k Genomes - Haematological Oncology

The 100K genomes project was launched by the Prime Minister late in 2012, and will be made up of 60K rare disease trios and 40K cancer and germline pairs. The aim of this project is to understand and create a new genomic medicine service for the NHS. In time this could translate to new and more effective treatments, improving patient outcomes, and the development of the largest genotype-phenotype database in the NHS which will be a ground-breaking resource.

The cancer arm also includes Haematological Oncology disease groups and the project opened for collection of these patient samples in 2017. Although Yorkshire and Humber GMC have been supplying rare disease patient samples for the 100K genomes project for some time, Leeds have now been given the go ahead to submit samples for the haem-onc arm of the project.

The haem-one arm will collect samples over 7 categories of disease:

*Chronic lymphocytic leukaemia
*Paediatric acute lymphoblastic leukaemia
*High risk myelodysplasia / acute myeloid leukaemia
*Unclassified haematological malignancies and unknown diagnoses
*Myeloma
*High grade lymphoma
*Chronic myeloid leukaemia

The eligibility criteria are quite strict and the amount of DNA required is more than the usual amount extracted at HMDS. Our plan is to work prospectively to identify potential patients from the outset, and save additional sample for future DNA and RNA extraction. The clinicians and GMC research nurses will then take forward the consent and submission of those patients eligible for the programme. Initially the work will involve patients from Leeds hospitals, but extend to others with an interest and enthusiasm to get involved with the project. We have already completed some work with Hull to create a pathway for patients from their sites.

HILIS functions we'd love you to use!

We would like all referrers to begin using the electronic request form which is available on the HILIS homepage (https://nww.hmds.leedsth.nhs.uk/hilis4). This form has been available to registered HILIS users for some time but we have now been able to modify this so that there is no requirement for a HILIS account. Use of this system could greatly reduce the potential for errors, from both the referral source and internally within HMDS, so please consider introducing this into your practice. For patients previously investigated by HMDS, a significant proportion of the request form can be auto-filled based on the NHS number, making the process much faster.

Many of you have already signed up to the report notification service. This sends an e-mail alert when new or updated reports are issued for your patients and is available to registered HILIS users with a @nhs.net account. More details about this can be found in a document entitled 'Report notifications in HILIS' on the HILIS homepage (address above).

New ALL diagnostic terms

A better understanding of the biology of T and B-ALL has allowed us to further classify many new cases of ALL, NOS into more refined categories. This is in keeping with the revised WHO classification of ALL.

You will now see these two new categories in your integrated reports:

B-ALL: B-lymphoblastic leukaemia (*BCR/ABL1*-like).

These are B-ALL which lack all known cytogenetic abnormalities but where activating translocations of genes have been detected by FISH. Although they lack the classical *BCR-ABL1* translocation, their molecular profile is very similar to *BCR-ABL1* positive ALL. Currently these are leukaemias with a poor prognosis but this might change in the future as new TKIs are incorporated in the treatment/maintenance of these cases.

T-ALL: T-lymphoblastic leukaemia (early T-cell precursor phenotype).

These are T-ALL with a characteristic phenotype lacking CD5, CD8 and CD1a but expression of one or more myeloid markers. Their genetic profile is similar to other myeloid malignancies and different from other T-ALLs. Although biologically different from other T-ALLs, impact on prognosis is still unclear, although many authors would argue in favour of this phenotype conferring a poor prognosis.

Sequencing variant annotation

Please note we have updated our annotation tool to improve the details for each variant. The new tool reports the genomic coordinates of small insertions and deletions using updated nomenclature. When comparing reports from the previous annotation tool, 1bp differences will be observed. The cDNA and protein coordinates remain unchanged. This does not affect the reporting on SNVs.

New consultant

We are pleased to welcome Dr Hebah Ali to HMDS to add to our consultant staff. Hebah has a background in histopathology & haematopathology, most recently working in the Specialist Integrated Haematological Malignancy Diagnostic Service at University College, London.

HMDS study day

Our study day this year is on Friday 29th June and again has a packed program, including a contribution from the Chief Scientific Officer, Professor Sue Hill. For more details and to register, visit **www.hartleytaylor.co.uk**.