Haematological Malignancy Diagnostic Service

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Welcome back

Welcome to the second HMDS newsletter. In this edition, we have highlighted two improvements in the diagnosis and management of myeloid malignancies. There has been huge increase in molecular technologies available to clinical laboratories and our recent implementation of high through-put sequencing (HTS) in the routine setting is particularly exciting. High throughput sequencing technology enables the analysis of multiple genes in parallel in large numbers of samples during one sequencing run. This technology has revolutionised molecular biology and has led to the identification of novel genes implicated in cancer pathogenesis. We will be applying HTS in the area of lymphoid malignancy in the near future and there will also be further advances with additional technologies over the coming year so watch this space….

Application of high through-put sequencing in myeloid disorders

Since September 2015, HMDS has been performing high throughput DNA sequencing in routine practice. We were one of the first laboratories in the UK to implement this technology and to date have analysed and reported over 1,000 samples. Our in-house designed panel targets the most frequently mutated genes across the spectrum of myeloid malignancies (Table 1) and we are currently performing this on all patients with a new or relapsed diagnosis of AML, MDS, MDS/MPN overlap syndromes or myelofibrosis. The ultimate goal of this test is to identify acquired variants in the DNA that may contribute to the diagnosis, prognosis or treatment of these patients.

Functional Pathway	Gene
DNA Methylation	TET2, DNMT3A, IDH1, IDH2
Chromatin Modification	ASXL1, EZH2
Splicing	SF3B1, SRSF2, U2AF1, ZRSR2
Transcription Factors	NPM1, RUNX1, BCOR, WTI, TP53
Signalling	FLT3, NRAS, KRAS, CBL, cKIT, JAK2, MPL, CSF3R, STAT3
Cohesin complex	STAG2
Other	SETBP1, CALR

Table 1. Panel of genes included in the HMDS myeloid panel

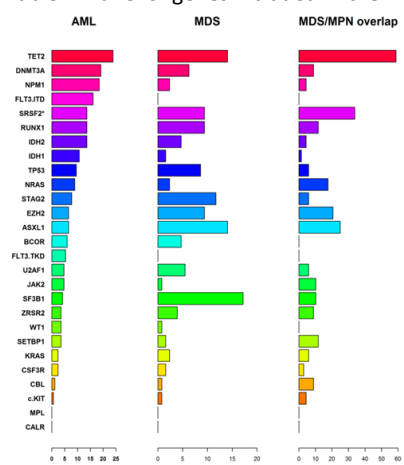


Fig. 1. Spectrum of mutations demonstrated in HMDS dataset

To date we have processed, analysed and reported 1,137 samples with a failure rate of only 2% and mutations detected in over 90% of cases. The spectrum of mutations reflects that reported in the literature (Fig.1).

Indications

There is an ever expanding literature on the prognostic impact of mutational analysis and several genes in the panel are targets of novel therapies in current clinical trials making this test highly applicable in those patients with a newly confirmed diagnosis.

The diagnostic utility of HTS is still an area of on-going research in HMDS but there is increasing evidence that the detection of a somatic mutation can provide evidence of clonality in difficult cases. Sequencing can be directly requested by referring clinicians for such cases for an additional charge, keeping in mind the following limitations:

- Although this panel covers many of the key genes implicated in this disease group, it is not a complete list and many low frequency genes are not covered.
- The panel does not cover the entire coding regions of many of the genes and is not designed to discover novel variants in a DNA sample.
- The panel is designed to detect somatic mutations and is therefore not suitable for constitutional or inherited variants. Any patient in whom a familial or inherited malignancy is suspected should be referred to clinical genetics for assessment.

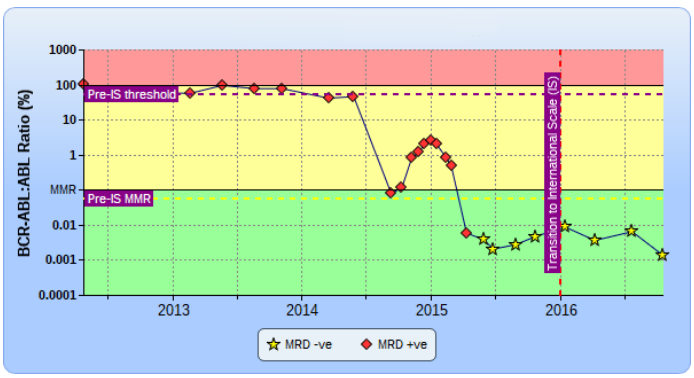
Paperless reporting

Following feedback from users about how our reports are most frequently viewed, HMDS is planning to stop printing and posting reports in the near future. To help us ensure that reports still get delivered successfully, we are asking users to supply a nominated nhs.net e-mail address for reports. This must be a generic, departmental e-mail, although copies will still be e-mailed to personal nhs.net accounts if requested. Please send details of nominated accounts to **hmds.lth@nhs.net**.

Improvements in the monitoring of CML

From the beginning of the year we have started to report CML RQ-PCR monitoring results on the International Scale (IS) using our local conversion factor (CF=0.58). This has been derived following a significant amount of work by Liz Wilkinson, involving a large number of sample exchanges between HMDS and Prof. Nick Cross at Wessex Regional Genetics Service in Salisbury.

Reporting on the IS allows direct comparison of results from different laboratories within the UK and further afield and is also a prerequisite for entry onto many of the on-going TKI de-escalation/cessation trials. The change will have a direct effect on patient data and all patients will see their BCR: ABL1 ratios fall. A further reduction will also be apparent graphically as the threshold for achieving a major molecular response (MMolR) will increase. Currently a 3 log reduction in transcript levels is calculated using our locally derived median presentation level of 55%. Reporting on the IS will increase the MMolR threshold from our current local level of 0.055% to 0.1%. For a period of six months the IS result will be reported in conjunction with our local result. The local result will still be plotted graphically and it is hoped that all referrers and patients will be able to see at least two samples over this period and how this change will affect their results. From 1st July 2017 all results will be reported graphically using the IS only and an example of how this will change the graph that is attached to each report is presented below.



CML monitoring requesting has been improved by the creation of an online request form available via HILIS (**Resources>Documents>HMDS BCR-ABL monitoring online request form**). The form can be populated using the appropriate NHS No if the patient has been previously registered on the HILIS system and will require the referrer to state the date the patient was diagnosed and a number of treatment related questions. This information will be used to provide clinically relevant information back to the referrer regarding achievement of optimal response at critical timepoints in a patient's treatment with reference to the current ELN 2013 guidelines. Online requesting will also alert the referrer if the patient has had a previous monitoring sample received in HMDS within the past 90 days, so avoiding over testing. Obviously this can be overridden if more frequent testing is required from a clinical context.

We are particularly concerned that a small number of patients fail to be monitored at regular intervals; this may be due to failure to attend planned out-patient appointments or a series of failed/inadequate samples received by the laboratory. We are in the process of setting up a regular monthly search on HILIS that will identify any patient that has not received an adequate monitoring result within the past 7 months. This list will be generated and interrogated by staff within the molecular section with an e-mail warning sent to inform the referring clinician of the issue. It is hoped that this system will be implemented in the next few months and will ensure that all patients receive appropriate regular monitoring of their disease.

HMDS study day

Registration is now open for the 2017 HMDS study day on Friday, 30th June. As in previous years, we have an interesting programme, including sessions on histiocytic disorders, T-cell malignancies, MDS/MPN overlap syndromes and mantle cell lymphoma.

Organisation of the meeting is been provided by Hartley-Taylor this year so for registration and other details visit their website: **http://www.hartleytaylor.co.uk**.

Farewell to Dr Andrew Jack

Having stepped down from his role as Head of Department within HMDS some time ago, Dr. Andrew Jack has now fully retired from the Leeds Teaching Hospitals Trust. We wish him all the best for his retirement!

To avoid any unnecessary delays, please ensure that letters and referrals for HMDS investigations are no longer addressed to Andrew.

Transport of tissue samples

Following several problems with the adequacy of tissue specimens recently, we would like to remind users of our guidelines regarding referral of tissue samples to HMDS.

All specimens should have a least one piece transported in fixative. The default if in doubt should always be to fix.

If there is sufficient tissue remaining, this can be sent fresh, which does allow a broader range of tests to be performed (for example, flow cytometry and more complex molecular analysis). The tissue should be placed on a saline-soaked swab but should **NOT** be immersed in saline. This applies to all specimen types, e.g. skin, core biopsies and endoscopic biopsies. Larger biopsies, such as whole lymph nodes, can be bisected to place a piece in formalin and keep a piece fresh.

All fresh tissue should be transferred to HMDS in the shortest possible time. Delays of **>1hr** result in severe degradation of the specimen and this is likely to compromise interpretation.