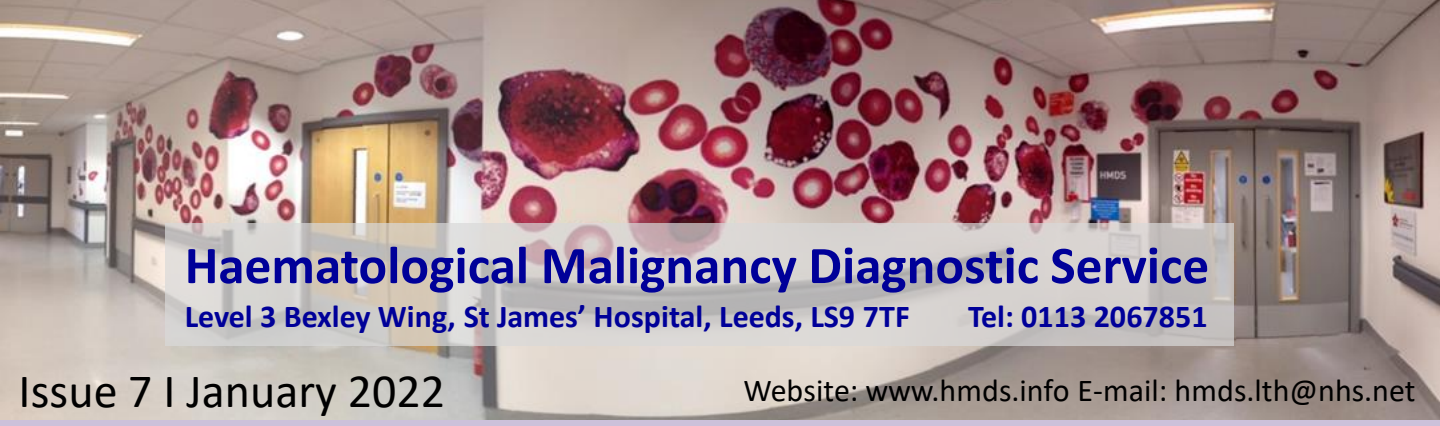

Haematological Malignancy Diagnostic Service

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Happy New Year

Huge apologies that it has been so long since we last published a newsletter. As I'm sure you can appreciate, the last 18 months or so have been very challenging and we have had to put a hold on much of our peripheral activity in favour of keeping the service going. Obviously, communication with our users is important to us so hopefully we will have more capacity for things like this in the coming year. A huge amount has happened since our last edition, in particular with respect to genomics testing, so the bulk of this newsletter will concentrate on that. As always, please get in touch if there is anything specific you think it would be useful for us to cover.

HMDS wishes all our users a Happy New Year and we look forward to working with you all again in 2022.

Update on provision of Genomics testing through HMDS

As all of you should now be aware, there has been a national reconfiguration of genomics testing which has been on-going for several years now. In our region, this now means all genomics is provided by the North East and Yorkshire Genomic Laboratory Hub service (NEY GLH), a collaborative effort involving several different laboratories in Leeds Teaching Hospitals NHS Trust (LTHT), Sheffield Children's NHS Foundation Trust (SCH) and the Newcastle Upon Tyne Hospitals NHS Trust (NUTH). Leeds has been designated as the Central laboratory for the GLH and the genomics/molecular staff from within HMDS form part of the central laboratory team. Clearly, the integrated nature of the service at HMDS is critical and this will be maintained. HMDS will continue to receive all haem-onc samples as we do currently and perform genomics testing as required.

The last twelve months have seen significant workload increases for HMDS as work has been centralised in Leeds from other parts of the GLH. This has had a particular impact on high-throughput sequencing and clonality, where we have seen large increases in the number of samples referred to us, but there has also been a huge amount of development work undertaken to fulfil the national genomics test directory. The molecular team have been working extremely hard under quite challenging circumstances to deliver a large amount of change and continue to keep up with service demand. Unfortunately, there has been some lengthening of turn-around times – we are aware of this and are doing as much as possible to resolve this. We have recently recruited additional laboratory staff, which will help in the long term. There is obviously a period of training and competency assessment which needs to be completed before this impacts fully but we are hopeful this will generate improvements over the coming months.

In the meantime, we will continue to prioritise clinically urgent requests, such as key targets for newly diagnosed acute leukaemia. If you have HTS or other molecular results which are required urgently please contact the lab on 0113 2067851 and discuss with the molecular team.

HMDS study day

Thanks to support from Hartley-Taylor, we ran a hugely successful on-line study day in September last year. We had 365 individuals who registered and logged in to participate in at least part of the day and for the first time we had several international delegates, which is fantastic. We are starting to plan our offering for this year – a decision is yet to made about whether this will be a face-to-face meeting or a virtual offering, but we will update you as soon as we know more.

The HMDS Annual Haematopathology Update Day

Incorporating Novel Diagnostics into Patient Management ...
Patient Pathways from Laboratory to Bedside

Wednesday 8th September 2021

VIRTUAL EVENT

Organisers: Dr Ruth de Tute, Professor Roger Owen and Dr Cathy Burton, *HMDS Leeds*

New consultant haematologist

A belated welcome to Dr Anita Sarma, who joined the HMDS team in the early part of last year. Anita is a haematologist who was previously based in London, at Kings and many of you will already have met her in MDTs and other meetings. Her current clinical focus is CLL/B-LPD's but Anita has a broad interest in the whole spectrum of haematological malignancies and is already proving to be a huge asset within the department.

Changes to myeloid high-throughput sequencing

Since 2015, HMDS has been providing high-throughput sequencing of myeloid malignancies using a targeted panel covering 26 genes reported to be frequently mutated across the spectrum of myeloid malignancies. The is an ever expanding literature on mutational analysis in myeloid malignancy, and other haematological neoplasms, and novel targets with potentially important prognostic or treatment significance have been identified since our original panel was developed. To incorporate these additional genes and also make improvements in sequencing for other haematological disorders, we have developed a bespoke panHaemOnc NGS panel designed to conform to NHSEs plan to provide large panel NGS testing and analysis for HaemOnc patients. The panel has been designed to cover all genes on the test directory (TD) for all haematological malignancies, plus a number of other genes of interest from a research and a future planning perspective. The design uses Twist technology and has 258 genes known to be implicated in HaemOnc disorders.

A bioinformatics pipeline has been modified and validated for use with this panel and the analysis will be bioinformatically managed as a series of virtual panels for disease groupings, which are currently:

* Acute and myeloid (myeloid malignancies and ALL)
* Lymphoid (lymphoma/mature B-LPDs)
* Histiocytosis
* Myeloma

We have so far gone live with processing and reporting for the Acute and myeloid panel. This has increased areas of coverage for many genes and has added a significant number of reportable genes to the previous myeloid panel:

ANKRD26, CEBPA, CUX1, DDX41, ETNK1, ETV6, FBXW7, GATA1, GATA2, GNB1, HRAS, IKZF1, KMT2C, NF1, NFE2, NOTCH1, PHF6 PPM1D, PTEN, PTPN11, RAD21, SH2B3, STAT5B, UBA1

The increased coverage of previously covered genes and the addition of so many new genes to the panel has meant that analysis and reporting time for each case has been significantly increased. A large amount of work goes into verifying and classifying variants which have not been seen by the laboratory before and the number of variants seen in each case has increased considerably. We acknowledge that this has increased the turn-around time beyond the 3 week target but are trying our best to reduce this. As the experience with this new panel grows, the reporting time will be reduced but please do let us know if you have a case you need urgently and we will do our best to prioritise.

The bioinformatics pipeline also allows detection of partial tandem duplications in *KMT2A*, which has been shown to be associated with adverse outcome in AML. We are currently still verifying these abnormalities with SNP array but will have sufficient data soon to report these directly from the sequencing panel.

The panels for other disease groups will be introduced as validation of analysis is completed. The panel will be applicable to both fresh and FFPE tissue, so more detailed sequencing of lymphoma tissue samples should be available within the next 12 months.

Current investigations for myeloid malignancy

Since there have been some changes to our testing pathways for myeloid malignancies recently, particularly with respect to genomics, we thought it would be worthwhile summarizing our current routine approach:

Suspected/new diagnosis of AML

Patient >60yrs old
FLT3 ITD
FLT3 TKD
NPM1
Should be reported within 48hrs of request
HTS sequencing (acute and myeloid panel)

Patient <60yrs old
FLT3 ITD
FLT3 TKD
NPM1
Should be reported within 48hrs of request
RUNX1-RUNX1T1
CBFbeta MYH11
HTS sequencing (acute and myeloid panel)

Suspected diagnosis of APML

PML immunofluorescence – within 24hrs
PML/RARA FISH
PML/RARA molecular

Other myeloid indications for HTS sequencing;
At diagnosis:
Acute undifferentiated or mixed phenotype acute leukaemia
MDS
CMML and other MDS/MPN overlap syndromes
MPN with myelofibrosis/primary myelofibrosis
At follow-up:
Clinical disease progression
Morphological evidence of change

Investigations for MPN:
PB: *JAK2/CALR* by fragment analysis
For patients with WT *JAK2/CALR, JAK2* exon 12 and *MPL* exon 10 can be performed.
Requests should meet the WHO guidelines for the diagnosis of MPN –

- Hb >165g/l or Hct >49% for males and Hb >160g/l or Hct >48% for males OR Plt ≥450 x 10^9/l
- Persistent and unexplained WBC leukocytosis or neutrophilia/monocytosis
- Morphological evidence of teardrop poikilocytes, left-shifted neutrophil series or Fe deficiency in context of normal RBC
- Relevant clinical details (known MPN on treatment, splenomegaly, suspected myelofibrosis, VTE)

FLT3 rapid testing

It is approximated that mutations of *FLT3* occur in 30% of AML patients, with internal tandem duplications (ITD) representing the majority. *FLT3*-TKD (tyrosine kinase domain) mutations have an incidence of 7-10% in AML (Daver et al, 2019).

FLT3-TKD (tyrosine kinase domain) mutations are small mutations in the activation loop of *FLT3*, mostly representing point mutations in codon D835 or deletions of codon I836 and, together with *FLT3* internal tandem duplications (ITD) they are one of the most frequently identified genetic alterations that have a role in leukaemia pathogenesis.

We have performed rapid testing for *FLT3* ITD for sometime but have recently added targeted assessment for *FLT3* TKD to our rapid approach.

Several *FLT3* TKIs – including the multikinase inhibitors midostaurin and sorafenib and the more-selective *FLT3* inhibitors crenolanib, gilteritinib, and quizartinib – are now available or currently being evaluated (Patnaik et al. 2018). The ELN guidelines recommend the results of *FLT3* testing should be made available within 48–72 h after the initial diagnosis of AML so that targeted therapy can be initiated in a timely manner (Dohner et al 2017).

Happy Birthday to us!

The HMDS service celebrates it's 30th birthday this year. When the department was established in 1992, it was the first integrated haemato-oncology service in the UK and initially had around 20 staff. Over the intervening years, we have grown and developed considerably and now have nearly 70 staff and deal with in excess of 40,000 cases per year. We are hoping to organize some events to celebrate this milestone later in the year so watch for details coming soon.