Conventional cytogenetic karyotyping and targeted FISH analysis have been the established standard of care in investigating chronic lymphocytic leukaemia. These methods whilst providing prognostic and response prediction are subject to a number of limitations including low level of resolution, failure to achieve the representative cell line in culture and the restrictions of targeted investigation by FISH. Whole genome microarray technology has now superseded conventional cytogenetics in the diagnosis of constitutional anomalies due its higher resolution. The technology has the added benefit of detecting copy neutral loss of heterozygosity (CNLOH) and complex rearrangements such as chromothripsis.

We performed an unbiased comparison of findings from SNP microarray using the CytoScan750K SNP Array (Affymetrix), conventional karyotype analysis and FISH on 50 CLL patients. The results illustrate a higher detection rate for pathogenic abnormalities in molecular karyotyping compared to conventional 40/50 (80%) and 22/50 (44%), respectively. The detection of additional pathogenic abnormalities not seen by microscopy accounted for 23/50 (46%) and CNLOH detected for chromosome 13, 20 and 22 in 7/50 (14%).

We demonstrate the benefits of SNP based whole genome profiling of CLL and propose its inclusion in the testing regime for the diagnosis and management of CLL patients.

MASSIVELY PARALLEL SEQUENCING IN COMPLEX BLOOD GROUP INVESTIGATIONS: RESOLVING THE PREVIOUSLY UNRESOLVABLE

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Analysis of all variants in 33/36 blood group systems for 29 samples with known extended genotypes was performed by massively parallel sequencing (MPS) (Illumina TruSight One Panel, data alignment with Human Reference Genome GRCh37). No discordant results with known extended genotypes were noted. Sequencing data was obtained for a further 19 research cases. Six samples required analysis of the homologous *RHD* and *RHCE* genes with further resolution using Sanger sequencing. This included one sample that phenotyped as very weak RhD positive and SNP typed as RHD(ex9:del/CE). MPS also indicated a RHD(ex9:del/CE) but was unable to discriminate between a deletion or a rearrangement with *RHCE*.

MPS provided additional phenotype predictions for seven samples referred to assist with complex antibody resolution. MPS identified rare/novel alleles in a further six samples and resolved a discrepancy between SNP typing and serology in favour of serology by identifying a rare null Jk allele. In another case the Cromer system was suggested as soluble CD55 inhibited the antibody; MPS identified a homozygous novel nucleotide change c.203G>A, predicting p.S68N. For novel uncurated mutations *in silico* tools were applied to predict phenotypic change.

In these examples, MPS provided a powerful tool to assist resolution of complex red cell serology.

HYBRID GLYCOPHORINS: SILENT GENETIC VARIANTS COMPLICATE GENETIC TESTING

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Characterisation of the genetic changes characteristic of hybrid glycophorins by SNV mapping, provides a reliable basis for prediction of the complex phenotypes associated with these clinically significant variants. In particular Gp.Mur is found at prevalences up to 10% of East Asian ethnic groups.

Use of genotyping to screen for and characterise these glycophorins is, however, complicated by 'silent' genetic change.

In one case of silent genetic change the 5' end of the BAB allele is in intron 2 and 3' end is in the beginning of pseudoexon 3. The insertion of GYPA sequence does not extend to the splice site at the 5' end of intron A3 and splice site of GYPB pseudoexon 3 is homozygous in the individual with this new BAB allele. As the pseudoexon remains silent, no is antigenic change is expected.

In a second case a BAB gene conversion typical of Gp.Mur was detected but a further genetic change resulted in inactivation of splice site at the 5' end of intron A3, preventing phenotypic expression of the hybrid exon.

To predict phenotype the use of sequencing as well as SNV mapping is required to specifically confirm the predicted phenotype.

REVIEW OF A PAEDIATRIC MASSIVE TRANSFUSION PROTOCOL IN A CHILDREN HOSPITAL

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Aims: Paediatric massive transfusion protocol (MTP) in our institution was introduced in 2013. It is divided into 4 codes based on patient's weight, which upon activation, facilitate the timely provision of red cells, platelets and plasma in a pre-determined ratio. We aimed to review the response time, blood product usage and wastage from MTP activations in our institution.

Methods: Transfusion records of paediatric requiring MTP activations from 2013 to 2015 in our institution were reviewed retrospectively.

Results: There were a total of 12 MTP activations since 2013. Median response time was 10 minutes (range 6 to >1 hr). MTP was ceased in cycle 1 for 8% of the patients, cycle 2 for 42%, cycle 3 for 33% and beyond cycle 3 for 8%. There were 8% of patients who did not fully utilise the blood products within cycle 1. 2 units of red cells, 1 unit of platelet and 1 unit of plasma were wasted in the 12 MTP activations.

Discussion: Paediatric MTP allows the provision of suitable blood products to bleeding patients. There is a need to fine-tune the activation protocol to reduce response time and blood wastage. Blood products within the MTP may be modified to include cryoprecipitate, to better support bleeding emergencies.