Fusion of ETV6 to FLT3 in a mixed phenotypic acute leukemia arising from myeloproliferative neoplasm with eosinophilia

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ETV6/FLT3 fusion gene has been recently reported in association with myeloproliferative neoplasm with eosinophilia (MPN-Eo) and peripheral T-cell lymphoma. Favorable clinical response to a targeted FLT3 tyrosine kinase inhibitor (FLT3 TKI) was noted.
Here we report a novel phenotype associated with *ETV6/FLT3* rearrangement, in a 38 year old female with presentation of progressive lymphadenopathy. Lymph node biopsy showed mixed phenotypic acute leukemia (MPAL) with expression of T-cell and myeloid markers. Bone marrow morphology was consistent with MPN-Eo and no evidence of MPAL. Karyotype analysis revealed 46, XX, t(12;13)(p13;q12). *ETV6/FLT3* fusion was demonstrated by *fluorescence in situ hybridization*.
This is the first report of *ETV6/FLT3* rearrangement showing a phenotype of extramedullary T/myeloid MPAL arising in the setting of MPN-Eo. We suggest addition of this entity to WHO category of "myeloid/lymphoid neoplasms with eosinophilia", particularly given the possibility of clinical response to FLT3 TKI in MPAL, a disease usually associated with poor prognosis.

Tales of two unusual cryptic cytogenetic 15;17 APL variants

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The 15;17 translocation that fuses the *RARA* gene on chromosome 17q21.1 to the *PML* gene on 15q24.1 is the hallmark of acute promyelocytic leukemia (APL). However, in 10% of cases, there are variant cytogenetic rearrangements resulting in the fusion of PML and RARA, most commonly 3-way translocations involving chromosomes 15, 17 and a third partner chromosome, or insertion of chromosome 17 material including the RARA gene within chromosome 15 at band q24.1. Insertion of chromosome 15 material including the PML gene within chromosome 17 has also been reported. We describe two unusual cryptic cytogenetic APL variants that were reported within one week of each other in the VGH Cytogenetics Laboratory. The cytogenetic-molecular characterization of these two variants will be presented. These cases highlight the importance of bone marrow morphology and FISH analysis for confirming the diagnosis on APL.

When you get more than you bargained for: FISHing multiple myeloma samples using a mini panel for high-risk cytogenetic anomalies

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Multiple myeloma (MM) is characterised by accumulation of clonal plasma cells in the bone marrow and is the second most common hematological malignancy after lymphoma. Chromosomal abnormalities are among the most important prognostic parameters for patients with MM, *IGH* rearrangements such as t(4;14) [FGFR3/WHSC1 & IGH] or t(14;16) [IGH & MAF] and deletions of the *TP53* locus being associated with an adverse prognosis while deletions 13q are often

associated with an intermediate prognosis. As of April 2013 the CHEO Cytogenetics Laboratory, in collaboration with The Ottawa Hospital, has been offering interphase FISH analysis on isolated plasma cells using probes for the detection of t(4;14) and *TP53* deletion. This MM mini-FISH panel is routinely offered to patients 60 years or younger with no significant comorbidities and in whom the plasma cells count in the bone marrow is 10% or higher before CD138 selection, as these patients would typically be eligible for a bone marrow transplant. Although data is still limited, an abnormal hybridization pattern was found in nearly 50% (9/19) of patients. Of these, only 2 patients had findings consistent with the targeted rearrangements [t(4;14) and deletion of *TP53*] and both cases showed an atypical abnormality. The abnormal hybridization patterns will be presented and implications for interpretation of results and analysis guidelines will be discussed.

Development of *TFE3* (Xp11.2) and *TFEB* (6p21) break-apart FISH assays on paraffin-embedded tissues to diagnose translocation-associated renal cell carcinoma (t-RCC)

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A subset of renal cell carcinomas that primarily affects children and young adults is associated with various chromosomal translocations. These t-RCC usually present at an advanced stage in children, and are associated with a relatively poor prognosis, especially in adults. However, these tumors can be a challenge to diagnose by morphology alone, and immunohistochemistry has poor reproducibility. The t-RCC may exhibit translocations involving *TFE3* on chromosome Xp11.2, which has multiple partner genes, and *TFEB* on chromosome 6p21 [t(6;11), *MALAT1(Alpha)/TFEB*]. FISH has been shown to have high specificity and sensitivity for translocations, including these in t-RCC. We have developed and evaluated two dual-color break-apart FISH assays to detect rearrangement of *TFE3* and *TFEB* in formalin-fixed paraffin-embedded (FFPE) tissues. Database validation was performed in three steps. First, probe localization was confirmed and analytic sensitivity and specificity was tested. Second, FISH with normal and abnormal RCC specimens was performed to establish the probe signal patterns and scoring criteria. Finally, 20 negative controls (tonsil specimens) were scored to establish the cut-off values. For the X-linked *TFE3*, two separate databases, male and female, were required. These studies confirmed *TFE3* and *TFEB* FISH tests are highly sensitive and specific for t-RCC, and especially useful for cases that have equivocal or negative immunohistochemistry results.

Translating genomics into improved care for children with brain tumours

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Brain tumours are the leading cause of cancer-related morbidity and mortality in children. One of the major goals of our translational research program at the Department of Pediatric Laboratory Medicine at The Hospital for Sick Children is to translate novel genetic information into improved diagnostic, prognostic and theranostic testing for pediatric cancer including

pediatric brain tumours. Pediatric low grade astrocytomas (PLGA) are the most common brain tumour of childhood. While rarely fatal, these tumours, if incompletely resected, have a high risk of recurrence. Deciding which children to treat, and at what point in their disease, is a major clinical quandry. Recent studies have demonstrated that the majority of PLGAs harbour mutations or fusion events involving the *BRAF* gene resulting in constitutive activation of the RAS/MAP kinase pathway. I will present data demonstrating the clinical implementation of this testing in our Department as well as the clinical significance of *BRAF* alterations for diagnosis of PLGA and for guiding treatment decisions for children with these tumours.

Integrative genomic analysis improves brain tumor diagnostics

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Genomic (copy number, mutational) profiling of formalin-fixed paraffin-embedded (FFPE) samples has the potential to improve cancer diagnostics, identifying characteristic aberrations relating to grade and/or histology; however, the use of this information in a CLIA setting is not yet routine. We sought to report our experience with the implementation of comparative genomic hybridization (CGH) and targeted sequencing across a series of >600 primary brain tumors. For glioblastoma multiforme (GBM), the most common and aggressive primary brain tumor, the clinical testing results of comparative genomic hybridization (n=236) and targeted mutation genotyping (n=94) were collected and compared to large, publically available, genomic datasets from fresh frozen tissue (i.e. TCGA). Across our cohort, clinically relevant loci were detected, including: 1p/19q co-deletion, *EGFR*, *EGFRvIII, PDGFRA, CDKN2A and PTEN*, as well as recurrent mutations in *IDH1*, *TP53 and PTEN*. Patients >40 years old showed a classic copy number profile for GBM, including polysomy 7, *EGFR* amplification, *CDKN2A* loss or *PTEN* deletion. Notably, the tumors of younger GBM patients (<40 years) showed a distinct copy number profile. In addition, this genomic approach can clarify medulloblastoma subgroups and help resolve histologic classification of mixed tumors. Combined use of copy number and mutation assays performed on FFPE samples in the clinical setting is feasible and can both improve diagnosis of primary brain tumors and efficiently replace use of singleton tests in clinical trials.

Beyond the karyotype: A pilot study comparing microarray platforms for the identification of clonal abnormalities in hematological malignancies

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Microarray analysis has dramatically changed the clinical cytogenetic service by improving rates of identification of aberrations in both the constitutional and prenatal settings. In 2013, the ACMG released their guidelines for microarray analysis in neoplastic disorders[1] and further to these recommendations, Kingston General Hospital (KGH) Genetics Laboratory is expanding its testing algorithm to incorporate microarray analysis for the investigation of selected neoplastic specimens. Here we present the results of a small pilot study comparing results from four microarray platforms for a variety of hematological malignancies. We will present cases from this pilot study, comparing conventional results with the microarray results from OGT cancer + SNP 4x180k, Bluegnome 4x180k CGH+SNP Cytochip, Illumina's CytoSNP 850k array and Affymetrix[1]s Cytoscan HD. We will review the depiction of clonal abnormalities with these platforms compared to conventional karyotype. Finally, we will review the algorithm for implementation of testing at KGH for microarray analysis for a subset of hematological malignancies.

References:
1. Cooley LD et al.; Working Group of the American College of Medical Genetics and Genomics (ACMG) Laboratory Quality Assurance Committee. American College of Medical Genetics and Genomics technical standards and guidelines: microarray analysis for chromosome abnormalities in neoplastic disorders. Genet Med. 2013 Jun;15(6):484-94.

Chromosomal Mosaicism – SNP arrays and other tools for detection

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Mosaicism for either partial or full chromosome aneuploidies is an important source of morbidity, implicated in a significant proportion of cases referred for cytogenetic analysis. Traditional methods of assessing chromosomal aneuploidy, including chromosome analysis and FISH, have been used for decades with well-established methods of determining test sensitivity. Entering the era of chromosomal microarrays has added another layer of complexity to the detection of mosaicism and brought further challenges in the determination and validation of test sensitivity. Array-based assays hold the promise of detecting very low levels of mosaicism. Key validation considerations including software tools, the percentage of aneuploidy cells, tissue distribution, the size of the segment and the probe coverage within the chromosome segment affected by aneuploidy will be discussed.

Group presentation on interesting cases with unusual or challenging observations

Group presentation
Organized by Josée Lavoie, PhD FCCMG

This presentation aims to raise general discussion on some interesting findings observed during the course of conventional or molecular cytogenetic investigations and to discuss testing strategies.

1- Are all D13S319 probes equal for detection of interstitial deletion 13q in CLL?

Dr. Melanie Beaulieu Bergeron, The Children's Hospital of Eastern Ontario

2- Trisomy 13/Trisomy 18 mosaicism in a 12 year old male
Dr. Abdul Noor, The Hospital for Sick Children

3- Monosomy 7 detected by microarray analysis in a child referred for developmental delay
Dr. Mary Shago, The Hospital for Sick Children

4- MYC fireworks
Dr. Tanya Gillan, Vancouver General Hospital

The path to some surprising results in a case of prenatal diagnosis

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A 42-year-old pregnant patient received an NIPT result of normal male. A subsequent ultrasound result at 18 weeks gestation indicated a female fetus. After Genetic counseling, amniocentesis was performed. Both the QF-PCR and FISH results were consistent with the NIPT results of a male fetus. A repeat ultrasound at 19 weeks gestation showed subtle ambiguous genitalia. Chromosome analysis revealed level III mosaicism for trisomy 15. At this point the patient terminated her pregnancy. To further characterize this result and its potential effect on the fetus, molecular studies confirmed maternal UPD, consistent with a diagnosis of Prader-Willi Syndrome. This case illustrates the importance of performing UPD studies in the case of low level mosaicism of chromosome 15. It also highlights that in some cases, multiple tests and technologies are required to arrive at the correct answer.

15q11.2 duplication encompassing only the UBE3A gene is associated with developmental delay and neuro-psychiatric phenotypes

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Due to the presence of low copy repeats (LCRs) in the chromosome 15q11-q13 region, it is prone to recurrent structural rearrangements including deletions, duplications and inversions. This region is also subject to genomic imprinting, thus associated phenotypes show parent-of-origin specific effect. Interstitial duplications of 15q11-q13 region with maternal imprints are associated with a wide spectrum of neuro-psychiatric disorders including, autism spectrum disorders (ASD), developmental delay, learning difficulties, schizophrenia and seizures. On the other hand, individuals

with duplications of the same region with paternal imprints are often phenotypically normal or in rare instances mildly affected. These observations suggest that a dosage sensitive imprinted gene or genes within this region underline the risk for neuro-psychiatric phenotypes. Recent studies have shown that UBE3A is the only gene within this region that is solely expressed from the maternal allele in mature neurons. To date, no case with duplication of only UBE3A has been reported previously, therefore contributions of this gene in 15q11-q13 duplication phenotypes remains unclear.
We present here a female patient with developmental delay in whom we identified a 129 Kb duplication in chromosome region 15q11.2 encompassing only the UBE3A gene. The duplication was found to be maternally inherited in the proband. We further tested the segregation of this duplication and it was found to be segregating with a neuro-psychiatric phenotype in four generations. Expression analysis in cultured fibroblast confirmed the over-expression of the UBE3A in the proband compared to age-matched controls. Our study, for the first time, shows the clinical features associated with over-dosage of UBE3A in humans, and underscores the significance of this gene in phenotypic consequences of the 15q11-q13 duplication.

Prenatal diagnosis of chromosome abnormalities, new testing strategies for changing indications

Vincenzo Cirigliano, Head of Molecular Genetics, Labco Diagnostics, Barcelona, Spain

It is not a long time ago when the use of maternal age, to select women eligible for invasive procedures, was complemented by measures of maternal serum markers and combined with ultrasound nuchal translucency measurement. The selection of groups at risk for aneuploidies reduced unnecessary invasive tests while improving detection rates. This prompted the introduction of targeted QF-PCR tests for rapid prenatal diagnosis of common aneuploidies and to provide prompt relief for parents in cases with a normal result. Large scale application of QF-PCR has than shown the test to be able to detect the great majority of chromosomal abnormalities in prenatal diagnosis and, in centres with careful monitoring of pregnancies, the molecular test is also in use to replace conventional cytogenetic analysis for lower risk patients. However, the great majority of invasive procedures as a result of screening for trisomies still reveal a normal result, while about 1 in 10 cases of trisomy 21 are going to be undetected. More recently, the extensive use of aCGH in prenatal samples widened the range of detectable copy number variants (CNVs) of clinical significance in both higher and lower risk pregnancies with otherwise normal karyotype; this also questioned the utility of routine screening tests, which are mainly only targeted to common trisomies.
Non-invasive prenatal testing for aneuploidies (NIPT) by sequencing cell free fetal DNA (cfDNA) in maternal plasma is again and greatly improving the efficiency of screening, demonstrating detection rates approaching 100% for autosomal trisomies, with less than 0,1% FP rate. Whether NIPT is best used before, after or to replace conventional screening tests, is under evaluation and its implementation in screening programs will mainly depend on costs. However, the number of women actually undergoing invasive procedure is already decreasing significantly, which was after all the main target to develop NIPT.
Expanded NIPT tests to include XY aneuploidies first and, more recently, a few microdeletions are already available despite being still controversial its inclusion in a screening test for the general population. Due to the lower frequencies of these conditions it is highly likely this will

cause a significant increase of the overall false positive rates, generating new indications for invasive procedures.
Prenatal diagnosis of chromosome abnormalities is going through a period of fast transition where new and efficient non-invasive screenings coupled with molecular diagnostic tests are improving pregnancy management while increasing the complexity of both pre and post tests counselling.

Prenatal microarray: Clinical experiences and panel discussion

Moderator:
Dr. James Stavropoulos
Speakers:
Dr. Elena Kolomietz
Dr. Marsha Speevak
Dr. Sébastien Chenier
Dr. Elizabeth McCready

As prenatal microarray is becoming widely adopted in Canada, there are important challenges in service delivery that warrant special attention. In particular, the interpretation and reporting of microarray results can have far-reaching effects on patients and their families. This session will provide presentations of clinical experiences and challenges associated with prenatal microarray, followed by a panel discussion.

Development of a Hereditary Spastic Paraplegia (HSP) gene panel using exome sequencing

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Recent years have shown major improvements in accuracy and cost of next generation sequencing (NGS), making this an attractive option in genetic diagnostics. The most common strategy employed thus far has been the development of targeted gene panels associated with a specific genetic disorder. The ability to interrogate a large number of genes in one assay has resulted in an increased diagnostic yield, with a decrease in cost as compared to Sanger sequencing. We present the development of an *in silico* gene panel for the diagnosis of hereditary spastic paraplegia (HSP) using exome sequencing. The *in silico* gene panel can be readily expandable as new genes are implicated in HSP, and this approach provides opportunities for translational research opportunities.

Discovery of novel variants with clinical significance in a research setting

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The advent of methodologies for scanning the human genome at high resolution has led to the identification of vast amounts of rare genetic variation with implications for disease gene

discovery and diagnostics. However, integration of the available evidence to support variant interpretation can often be challenging, especially when the associated phenotype displays extensive clinical and genomic heterogeneity. Using recent advances in Autism Spectrum Disorder (ASD) genetics as a paradigm, this presentation will focus on the strategies used to establish the clinical significance of rare variation. We are performing a comprehensive genomic analysis of our Canadian ASD cohort by combining high-resolution SNP microarray genotyping with whole exome sequencing (WES) for detection of copy number variants (CNVs) and sequence level variants, respectively. In addition to discovery of new candidate genes we are assessing the detection rate of clinically relevant genetic variants. Our combined genomic analysis suggests that more than 30% of cases have a genetic variant that is likely to be associated with ASD or accompanying clinical symptoms. The interpretation and classification of rare genetic variation will be discussed.

Development of a clinical genome-wide DNA methylation test

Bekim Sadikovic and Guillaume Pare
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Gene-specific defects in DNA methylation are associated with constitutional disorders and cancer. Single-locus clinical DNA methylation tests are available for a number of epigenetic defects including imprinting disorders, uniparental disomy syndromes and certain cancers. Furthermore, disruption of DNA methylation has been shown to underlie a growing number of constitutional disorders such as prenatal exposure syndromes, overgrowth syndromes, chromatin remodeling and DNA methylation complex mutations and others. Nonetheless, no genome-wide clinical methylation test is currently available. The overarching aim of this project is to develop a clinical platform for genome-wide methylation analysis using the Illumina Infinium450K arrays and to assess its clinical diagnostic utility. Specific objectives are: (1) to develop a custom bioinformatic pipeline for methylation analysis and (2) to determine the percentage of pediatric patients with developmental and intellectual delay spectrum phenotypes that can be explained by aberrant DNA methylation of Mendelian genes. In this talk I will present the validation and our preliminary findings including first 500+ patients and discuss database and informatics pipeline development.

Small changes make a big difference: Improving efficiency and quality through automation of G-banding

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To increase cost effectiveness in a changing health care climate, our lab sought to eliminate redundancy and improve productivity in the G-banding process. The current manual banding process requires one full time technologist. Recognizing that efficiency could be improved in this area, we developed a procedure that would utilize automation focusing on trypsin time and environmental conditions as well as rearranging processes upstream of slide preparation. The laboratory analyzed 46 cases in this method comparison study which included two specimen types, bone marrow and peripheral blood. This accounted for several culture types used for specific cell lineages, giving a total of 105 slides paralleled. Corresponding slides were examined

by technologists using qualitative and quantitative assessments like mitotic index and banding quality. This study showed that humidity levels between 18%-38% were most favorable with the banding procedure tolerable up to 55%. The optimal temperature ranged from 18°C-24°C. Culture types were dropped and aged concurrently therefore eliminating another variable impacting by the trypsin treatment. When environmental conditions remain consistent the trypsin time was directly proportional to cell concentration. Furthermore, we found that automation improved banding consistency and efficiency, and increased throughput. The ability to automate a staining procedure resulted in a 64% decrease in the banding time, equivalent to approximately 0.4 full time technologists per year. With over 3000 cases in 2013, the additional time was allocated to process improvements in other areas of the laboratory and test development.

Direct transcriptional consequences of somatic mutation in cancer

Adam Shlien PhD, Associate Director, Translational Genetics, Department of Paediatric Laboratory Medicine, The Hospital for Sick Children

The disordered transcriptomes of cancer encompass direct effects of somatic mutation on transcription; co-ordinated secondary alterations in transcriptional pathways; and increased transcriptional noise. To catalogue the rules governing how somatic mutation exerts direct transcriptional effects, we developed an exhaustive bioinformatics pipeline for analyzing RNA-sequencing data, which we integrated with whole genome sequencing from 23 breast cancers. Using X-inactivation analyses, we find cancer cells are more transcriptionally active than intermixed stromal cells. 14% of 4234 genomic rearrangements caused transcriptional abnormalities, including exon skips, exon reusage, fusion transcripts and premature poly-adenylation. We found productive, stable transcription from sense-to-antisense gene fusions and gene-to-intergenic rearrangements, suggesting that these mutation classes may drive more transcriptional disruption than previously suspected. Systematic integration of transcriptome with genome data therefore reveals the rules by which transcriptional machinery interprets somatic mutation.

Clinical validation and comparison of custom NGS panels for the BRCA1/2 gene sequencing

Bekim Sadikovic, Barry Eng, Hanxin Lin, Ron Carter, and John Waye
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Next generation sequencing (NGS) is rapidly displacing Sanger sequencing in the clinical arena. While offering a potential for decreased cost, technological time, and possibly increased sensitivity compared to Sanger, NGS comes with its own set of unique challenges which include: data storage solutions, informatics pipeline development, and most importantly development and design of clinical assays meeting the diagnostic parameters of the current gold standard Sanger sequencing. We developed a custom automated clinical-grade NGS data storage and informatics pipeline, and tested four unique custom BRCA1/2 NGS library chemistries including Illumina Custom Amplicon, Agilent Haloplex, Nimblegen SeqCap EZ Choice, and Kailos in combination with Illumina MiSeq sequencing. We compared the four chemistries in regards to sensitivity and specificity for detection of 220+ Sanger validated variants form 50 clinical samples including

SNPs, small and large insertions, deletions and in/dels. We compared the sequence coverage performance for each chemistry, and performed validation of NGS copy number detection for 50 additional clinical samples with whole exon deletions/duplications. Here we present findings from this study, and describe our selected clinical BRCA1/2 NGS pipeline.

Clinical utility of the new cytogenetic/cytogenomic tools in reproductive medicine

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Columbia University Medical Center & the New York Presbyterian Hospital

Cytogenetic studies have had a major impact on reproductive medicine, yielding a greater understanding of the frequency of chromosomal abnormalities that occur during gametogenesis, embryonic development and pregnancy. Chromosome abnormalities account for approximately 65-70% of first trimester miscarriages with aneuploidy accounting for the majority of the cases. Aneuploidy has been found to be relatively common during fetal life, prompting the need for prenatal testing which has now been available for over 40 years. Traditional cytogenetic analysis has been the gold standard for assessing fetal karyotypes and has an accuracy of greater than 99% for detecting microscopically visible chromosomal abnormalities such as aneuploidy and structural abnormalities. With the recommendation in 2010 by the ACMG to replace karyotyping with chromosomal microarrays as the 'First-Line' postnatal test, attention has shifted toward assessing the utility of microarrays in a prenatal setting. The effectiveness of microarrays has also been assessed in the preimplantation stage where aneuploidy rates are believed to be even higher than at the fetal stages of development. The introduction of microarrays into the clinical arena has shifted the way we look at chromosomes to a genomics-based view, offering greater resolution and new diagnostic categories such as UPD. Cytogeneticists are now being referred to as cytogenomicists and molecular techniques are quickly becoming a part of cytogenetics standard operating procedures. This talk will present current experience with the newer cytogenomic technologies that are being utilized for diagnostics in both the preimplantation and fetal stages of development.