The role of FLT3 in sole trisomy 8 acute myeloid leukemia

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FLT3 (fms-related tyrosine kinase 3) with tandem internal duplication (ITD) is a known adverse prognostic factor in normal karyotype acute myeloid leukemia (AML). Since we recently observed several patients with trisomy 8 (+8) and FLT3 positivity, we asked whether FLTD ITD carried any prognostic significance in sole trisomy 8. Trisomy 8 is the most frequent numerical aberration in AML, occurring in 5% of cytogenetically abnormal AML as the sole chromosome change. The prognostic impact of +8 as a sole aberration remains unclear and may be classified either within intermediate- or high- risk groups.

We identified 37 adult AML patients (25 males : 12 females) with sole trisomy 8 treated at Roswell Park Cancer Institute between 1991 and 2008 with adequate follow-up and samples available for analysis. Treatment differed according to available protocols at the time.

FLT3 ITD was detected in 7/37 patients. FLT3 D835 was detected in 5 of 30 patients without FLT3 ITD but was not detected in any of the 7 FLT3 ITD-positive patients. Nucleophosmin (NPM1) was detected in only one case (FLT3 ITD/D835 negative). The presence of FLT3 ITD was associated with younger age and presence of trisomy 8 in all analyzed metaphases when compared to those without FLT3 ITD. AML patients with trisomy 8 and FLT3 ITD had similar complete remission and progression-free survival as those without FLT3 ITD.

Genetics of dermatofibrosarcoma protuberans family of tumors: from ring chromosomes to tyrosine kinase inhibitor treatment

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Dermatofibrosarcoma protuberans (DFSP) is a rare slow-growing infiltrating dermal neoplasm of intermediate malignancy. At the cytogenetic level, DFSP cells are characterized by either supernumerary ring chromosomes or t(17;22) that are most often unbalanced. Both the rings and linear der(22) contain a specific fusion of *COL1A1* with PDGFB. Whereas rings have been mainly observed in adults, translocations have been reported in all pediatric cases. DFSP is therefore a unique example of tumor in which (i) the same molecular event occurs either on rings or linear translocation derivatives, and (ii) the chromosomal abnormalities display an age-related pattern. In all DFSP cases that underwent molecular investigations, the breakpoint localization in PDGFB was found to be remarkably constant in exon 2. In contrast, the *COL1A1* breakpoint was found to be variably located within the exons of the alpha-helical coding region (exons 6-49). No preferential *COL1A1* breakpoint and no correlation between the breakpoint location and the age of the patient or any clinical or histological particularity have been described. Congenital case as well as pediatric or adult case presented all the same *COL1A1*-PDGFB rearrangement. The *COL1A1*-PDGFB fusion is detectable by multiplex RT-PCR or dual color FISH experiment adapted to fixed and paraffin embedded tissues. In less than 10% of DFSP cases, the *COL1A1*-PDGFB fusion is not found, suggesting that genes other than *COL1A1* or PDGFB might be involved in a subset of cases. We identified a DFSP without the *COL1A1*-PDGFB fusion gene. From a pathobiology point of view, it has been demonstrated that PDGFB acts as a mitogen in DFSP cells by autocrine stimulation of the PDGF receptor. The inhibitory

effects of the PDGF receptor tyrosine kinase antagonist have been demonstrated in vivo, and such targeted therapies is now an option for treatment of the few DFSP cases not manageable by surgery.

Case reports - Five rare clonal chromosome anomalies in seniors with myeloid disorders

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Five rare clonal chromosome anomalies found in five seniors with myeloid disorders are reported here. The first case is a 72-year-old male with chronic myelomonocytic leukemia-2 (CMML-2), having a sole chromosome anomaly of del(1)(p34p36.1), which was only reported previously in a case of acute myelocytic leukemia type M1 in the French-American-British (FAB) classification scheme. The second case is a 64-year-old male with refractory anemia with excess blasts-2 (RAEB-2), having clonal gain of a der(1)t(1;19)(p13;p13.1), which was only reported in a total of 16 cases with a variety of myeloid disorders. The third case is a 69-year-old male with acute myeloid leukemia without maturation, having a der(3)t(3;15)(p21;q21) and a dic(5;15)(q13;q21) due to the breakage of 15q at band 15q21. The chromosome complement of this abnormal clone is monosomy from 3p21 to 3pter, and monosomy from 5q13 to 5qter, which is equivalent to having a 5q deletion at the breakpoint of 5q13. The fourth case is a 79-year-old male with acute monoblastic/monocytic leukemia, having inv(11)(p15q22), which results in the rearrangement of NUP98 and DDX10, and was only reported in 8 cases as a sole chromosome anomaly. The fifth case is an 82-year-old female with refractory cytopenia with multilineage dysplasia (RCMD), having del(11)(q23) without cryptic MLL rearrangement, which was reported to be predominantly associated with primary myelodysplastic syndrome.

Her2neu amplification is strongly associated with 1p/14q co-deletion in recurrent meningiomas

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Meningiomas are common intracranial tumours in adults which are divided histologically into WHO Grades I-III, with Grade I regarded as benign, Grade II atypical and Grade III anaplastic. The 5-year recurrence rate is 7-25% for benign, 29-52% for atypical, and 50-95% for anaplastic tumours. The current methods to predict recurrence rely on histological criteria, such as histological subtype, mitotic index, cytological atypia as well as brain invasion. However these methods are not sufficient to competently predict recurrence, especially in benign tumours. Deletions of 1p and 14q have previously been reported to correlate with poor prognosis in terms of either recurrence or higher histological grades.Her2neu (ErbB2)

amplification is associated with higher grades and recurrence. This study identifies the cytogenetic differences between 22 recurrent and 25 non-recurrent meningiomas of all grades, utilizing FISH probes for 1p36, 14q11.2 and 17q11.2-12 on paraffin embedded tissue from the Brain Tumour Tissue Bank, London Health Science Center. We show a positive association for meningioma recurrence correlated with 1p36 deletion plus or minus 14q 11.2 deletions in all grades of meningiomas. Her2neu amplification coincides with 1p/14q deletions and is found in the higher grades of recurrent meningiomas.These genetic markers will be useful for predicting which patients are at increased risk for relapse regardless of the tumour grade.
Keywords: meningioma, recurrence, FISH, deletions 1p, 14q, Her2neu amplification

HER2 ISH: Challenging diagnostic cases

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With the introduction of HER2 targeted therapies, in particular HerceptinTM, HER2 status has become a critical component in selecting the best treatment options in both early and advanced breast cancer. The use of fluorescence *in situ* hybridisation (FISH) and other bright field in situ hybridisation (ISH) based methods, has become widely accepted as the gold standard in determining HER2 amplification status and in most cases provides a clear answer as to whether a case is amplified; however there are small subsets of cases that do not conform to conventional diagnostic guidelines. These anomalies take many forms and include heterogeneity, loss of signals, variations in signal size, high copy numbers of HER2 and CEP 17 and co-localisation of HER2/CEP17 signals. These raise questions about the underlying cytogenetic changes and their implications in the determination of HER2 status. The presentation will discuss evidence based or consensus based reporting guidelines along with the need for future research. Additional supporting molecular data will be presented where appropriate.

Cytogenomic aspect of morphologic acute promyelocytic leukemia without the classic t(15;17); diagnostic implication

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This is a brief review of recent development in molecular cytogenetics of morphologic acute promyelocytic leukemia (APL) lack of the classic translocation t(15;17)(q24;q21). APL is characterized by a proliferation of blasts and morphologically abnormal promyelocytes in the bone marrow and peripheral blood. A vast majority (~92%) of APL cases have G-banding detectable t(15;17)(q24;q21) leading to PML/RARA fusion. Diagnosis of APL requires demonstration of rearrangement involving RARA (17q21). The remaining t(15;17) negative APL-like cases may have 1) cryptic PML/RARA rearrangement; 2) no RARA rearrangement; 3) double minutes and MYC amplification; 4) translocation variants with a fusion of RARA to one of the partner genes: PLZF (or ZBTB16) at 11q23, NUMA1 at 11q13, NPM1 at 5q35, STAT5B at 17q21, PRKAR1A at 17q24, FIP1L1 at 4q12 and BCOR at Xp11; or 5) other translocations with novel

gene fusions (e.g. t(11;12)(p15;q13); NUP98/RARG). Patients with PML/RARA, NPM1/RARA and NUMA1/RARA respond to treatment with all-trans retinoic acid (ATRA) while those with PLZF/RARA and STAT5B do not.

This presentation emphasizes the significance of use of cytogenomic methods (e.g. FISH, RT-PCR, microarray or sequencing) for investigation of the t(15;17) negative APL-like cases. It will also discuss interesting cases from our Cytogenetics Lab.

Discrepancies between standard and molecular cytogenetics or molecular biology in haematologic cancers

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Diagnostic tools for haematologic cancers include standard (karyotype) and molecular (FISH) cytogenetics, as well as various data from the molecular biology and haematology laboratories. When it comes to cytogenetics, there is a great deal of variation between laboratories regarding culture length of specimen and the number of rearrangements that are looked for by FISH. In this talk, we will present cases of haematologic cancers for which discrepancies were observed between standard and molecular cytogenetics or molecular biology during the course of our investigations. First, we present cases in which a classic cytogenetic translocation was seen on karyotype, but for which confirmatory FISH analyses demonstrated that it did not implicate the characteristic genes. Then, we present cases for which we observed a negative selection of the abnormal cells, even after a short culture of 24h. Indeed, discrepancies were observed between results obtained by FISH after a 24h culture and the abnormal cell count reported by the haematology laboratory. However, a second FISH analysis on blood smears showed perfect correlation with the haematologic data. Altogether, these cases illustrate the importance of doing confirmatory FISH analyses of karyotypic findings, even when a seemingly pathognomonic rearrangement is seen. It also reinforces the necessity of doing such FISH analyses on specimen that spent as little time in culture as possible, preferably blood smears or direct preparations. Finally, these cases also remind us that the haematology and molecular biology laboratories can provide useful informations that need to be integrated in cytogenetic analyses, such as the DNA index, RT-PCR and abnormal cell count and morphology of bone marrow specimen.

CD13 expression is associated with favourable cytogenetics risk groups, and has an independent adverse prognostic effect in adults with Philadelphia chromosome negative B cell acute lymphoblastic leukemia

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Background. In adults with precursor-B lymphoblastic leukemia (BCP-ALL) a number of factors have been identified as being of prognostic value, including age, baseline white blood count (WCC) and cytogenetics. Although over 50 % of patients fall within the relatively favourable or

non-high-risk cytogenetic groups, only some of these patients will go on to obtain sustained remissions, while many others will relapse and die from their disease.
Methods. Immunophenotypic and cytogenetic factors, and their interactions, were analyzed in 126 consecutive adults with BCR-ABL negative BCP-ALL who were treated with a pediatric-based protocol at a single institution over a ten year period.
Results. In addition to age and white blood cell count (WCC), the following factors were found to be independently prognostic in our cohort: hypodiploidy / near triploidy, normal karyotype, expression of at least 1 myeloid marker, and CD13 expression. A novel association between CD13 expression and favourable cytogenetics was identified; moreover CD13 expression had an adverse effect on prognosis for patients with favourable cytogenetics, as well as for the entire cohort. Controlling for age, WBC, and cytogenetic findings, CD13 expression was an independent poor prognostic indicator for all 3 endpoints: overall survival (OS, p=0.049), event-free survival (EFS, p=0.013), and relapse-free survival (RFS, p<0.001). A proposed risk model for adults with BCP-ALL that includes 4 high-risk factors: age > 60 years, WCC > $30x10^9$/ L, SWOG high/very high risk cytogenetics and CD13 positivity, performs better than a risk model of cytogenetics alone for stratifying patients by OS (p=0.001), EFS ($p=7x10^{-4}$) and RFS ($p=8x10^{-4}$).
Conclusions. Our findings indicate that CD13 expression is associated with favourable cytogenetics risk groups in adults with BCR-ABL negative BCP-ALL, and has an independent adverse prognostic impact for all patients including those with favourable cytogenetics. Incorporating CD13 into a scoring system which also includes age, WCC and cytogenetics provides high discrimination for relapse risk and survival.

Detection of chromosome abnormalities using cytoplasmic immunoglobulin staining and FISH (cIg FISH) in multiple myeloma

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Multiple myeloma (MM) is the second most common haematological neoplasm in North America. It is characterized by the clonal expansion of genetically transformed plasma cells in the bone marrow and by the production of monoclonal immunoglobulins. Due to the low proliferation activity of MM cells *in vitro*, and the patchy nature of the disease, conventional cytogenetics and FISH analyses are hampered by normal cell contamination, resulting in a low detection rate in routine diagnostic testing. Therefore, identification of plasma cells is critical for an accurate result reflecting the plasma cell population in the bone marrow. Cytoplasmic immunoglobulin staining in combination with FISH (cIg FISH) enables detection of specific abnormalities in plasma cells. The validation results of cIg FISH from the Cytogenetics Laboratory at North York General Hospital showed an increased detection rate of 21.4%, 124.8%, and 49.7% for t(4;14), D13S319/LAMP1 and TP53, respectively, with an overall detection rate of 71%, compared to 42% by conventional FISH.
In conclusion, cIg FISH improves test sensitivity and specificity, especially when plasma cell levels are low in the total bone marrow cell population. It yields a better overall detection rate for chromosomal abnormalities in multiple myeloma.

Chromothripsis: fashion or fact- some considerations based on recent findings in osteosarcoma oncogenomics

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Chromothripsis-defined primarily by next generation sequencing (NGS) methods, encompasses small-scale DNA copy number changes and extensive intrachromosomal rearrangements that are restricted to a single chromosome or chromosome arm. Several recent NGS reports of diverse cancer types have shown that massive yet spatially localized genomic rearrangements have taken place and it is thought that these alterations may represent large-scale repair following a single catastrophic event. Detailed inspection of breakpoint regions in these studies has shown a series of simultaneous double stranded DNA breaks consistent with local pulverization of chromosomes. Fusion of the resulting chromosomal fragments are thought to involve non-homologous end-joining as the junction points have been shown to display limited or no homology, often with small insertions and deletions. The chromothripsis mechanism appears to run counter to established progressive chromosomal mechanisms such as the breakage-fusion-bridge and FoSTeS mechanisms. In this presentation chromothripsis will be briefly reviewed and considerations for embarking on NGS studies to better define complex aberrations restricted to a chromosome arm in the tumour osteosarcoma will be addressed.

My cutoff is bigger than yours! Experience from the BC inter-laboratory FISH validation study

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Fluorescence in situ hybridization (FISH) detects recurrent chromosomal abnormalities, which carry important prognostic significance in chronic lymphocytic leukemia (CLL) and is an essential component of patient care. In the province of British Columbia, population 4.5 million, CLL patients receive uniform evaluation and therapy based on centrally derived protocols. CLL FISH testing was implemented in 2006 and is performed at one of three cytogenetic laboratories in BC: Vancouver General Hospital, BC Cancer Agency and Royal Columbian Hospital. As differences in probe design, normal cutoff values, scoring criteria and reporting may differ between laboratories we sought to validate CLL FISH testing between the three laboratories to ensure standardization of results. Detailed interviews were conducted with each laboratory and nine previously tested blinded CLL specimens, with multiple FISH abnormalities, were tested in each of the three labs using standard institutional protocols. The inter-laboratory FISH validation study revealed concordant results for the majority of samples/FISH probes; however, four potential false positive and two potential false negative results, the frequencies of which were at or near laboratory cutoffs, were observed. Given these findings, a more conservative universal cutoff value of 10% may be considered to improve consistency between labs and minimize false positive or negative results. FISH standardization between collaborative organizations that work together in the care of CLL patients is essential to ensure valid results when pooling data for clinical or research purposes.

Challenging situations in the Cytogenetic Laboratory

Group presentation
Organized by Josée Lavoie, PhD FCCMG

This presentation aims at raising a general discussion on some interesting findings observed during the course of conventional or molecular cytogenetic investigations, to discuss testing algorithms and to highlight some cytogenetic observations that could have been misinterpreted or overlooked.

1. Diagnostic dilemma Never a simple answer.
   Agnes Wozniarski, Mont-Sinai Hospital.
2. A probable false finding on array CGH due to inter-chromosomal sequence similarity.
   Mary Ann Thomas, Alberta Children's Hospital
3. An interesting mantle cell lymphoma case that points out the potential for a missed diagnosis.
   Erica Dafoe, Michael Crump, Hong Chang, Adam Smith and Kenneth J. Craddock, University Health Network
4. Chromosome breakage test in Fanconi anemia: atypical results.
   Fléchère Fortin, Montreal Children's Hospital
5. "Jumping" (translocations) to AML.
   Barnaba Werunga, Saint John Regional Hospital, Horizon Health Network, New Brunswick
6. Chromosome 5p13.3 microdeletion: Report of a case with developmental delay and clinodactily.
   Maisa Yoshimoto, Julie Richer and Elizabeth McCready, Children's Hospital of Eastern Ontario
7. Testing strategies in multiple myeloma.
   Josée Lavoie, Montreal Children's Hospital

Please join us next year and share your valuable story!

Different rearrangements of the same chromosome in chorionic villi and amniotic fluid from the same pregnancy: how would this happen?

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A 33 year-old pregnant woman underwent CVS sampling at 12 weeks gestation because of a positive first trimester screen for Trisomy 21. Of the six fully analysed metaphases, five were tetraploid and one was diploid. The diploid cell had an unbalanced female karyotype with one normal chromosome 9 and a large abnormal 9. The tetraploid cells all had only two copies of the normal 9, a large abnormal 9 identical to the one found in the diploid cell, and a small marker chromosome of unknown origin. Assessment of all available metaphases did not identify any other diploid cells and all tetraploid cells examined carried the same abnormal 9 and the same marker. No karyotypically normal cells were observed.

FISH studies were performed to further interrogate the abnormality. The abnormal 9 had a single centromere (CEP9+), two signals for 9qh probe (D9Z3++) suggesting that this abnormal 9 is derived from an unbalanced translocation between the short arm of one 9 at band p24 and the long arm of the other 9 at band q12 – der(9)t(9:9)(p24;q12). The marker had a small signal for the 9 centromere probe (CEP9+) with no evidence for 9qh material, thus making it a second very small derivative 9.

A follow-up amniocentesis was performed and showed an unbalanced female karyotype with one normal 9 and one abnormal 9 that is different from the CVS study. This abnormal 9 had a terminal deletion in the short arm at band p22, which was verified independently by FISH and array CGH. Chromosome studies were performed on the parents and both had a normal karyotype.

The abnormal karyotypes in cultured CVS and amniocytes are presumed to be related given that both involve a rearrangement of chromosome 9 with a breakpoint at a similar location. However, it is not known whether one derived from the other or if both abnormal karyotypes derived via two different stabilizing rearrangements of a precursor abnormal cell type. A detailed discussion will be provided in the oral presentation.

Retrospective evaluation of high density genomic microarray analysis in the constitutional setting

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Introduction: The trend of genomic microarray platform design has been towards higher resolution and inclusion of allelic differentiation. With this higher resolution comes a concern for increased detection of abnormalities requiring increased time for interpretation. However, with the rapid rate at which new genes or regions are associated with clinical conditions, a high resolution whole genome platform is desirable as it is more likely to have adequate coverage over these newly identified regions.

Objective: Evaluate the performance of a new high density genomic microarray platform in patients with indications including intellectual disability, autism, and/or multiple congenital anomalies.

Method: Over 800 cases were evaluated using the Affymetrix Cytoscan HD platform, in-house developed filters, and the ChAS software. Imbalances were evaluated for deletions >50kb and duplications >400kb, with a second-tier evaluation of clinically relevant genes/regions at approximately >15kb. Considered factors included pathogenic and unknown detection rate, data-filters, interpretation tools, number of calls, evaluation of absence of heterozygosity and data management.

Conclusion: The detection rate for pathogenic versus unknown CNVs was 13% vs 12% respectively. Use of a tiered filter within the software allowed for higher resolution analysis of regions/genes of recognized pathogencity vs whole genome and allowed for addition of new regions without modifying the platform. The average number of calls per case was 5-6. The addition of allelic differentiation led to the detection of 5 UPD cases and allowed for

autozygosity mapping in other cases. The ability to link-out to various databases and embed annotations within the software improves interpretation.

A clinical algorithm for efficient, high-resolution cytogenomic analysis of uncultured solid tissue samples

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Purpose: Cytogenetic analysis of solid tissue is indispensible in perinatal care, reproductive planning, and detection of gestational trophoblastic disease. Unfortunately, methods in common use suffer from drawbacks related to cell culture and other factors. We propose a new diagnostic algorithm based on direct genetic analysis of tissues (without cell culture) using QF-PCR and array CGH.
Methods: Study samples consisted of specimens submitted to the cytogenetics laboratory between January and June of 2011 that were split and analyzed in parallel by our traditional algorithm (culture and G-banding, plus a FISH aneuploidy panel for culture failures) and the proposed "no-culture" algorithm (first line QF-PCR, plus array CGH on normal QF-PCRs). Data on cost and turnaround time were collected.
Results: Forty specimens were included. The algorithms produced results that were fully concordant in 22 cases, partially condordant in 9 cases, and discordant in 9 cases. The "no-culture" algorithm resulted in increased accuracy of results, a reduced cost per-specimen ($52 CAD) and per-diagnosis ($94), and improved turnaround time with virtually all cases reported per guidelines.
Conclusion: These striking results favor the "no-culture" algorithm, which we propose as a new gold standard for the clinical analysis of perinatal solid tissue specimens.

Keywords: oligonucleotide array sequence analysis; quantitative fluorescent polymerase chain reaction; products of conception; gestational trophoblastic disease

A Case of false-positive trisomy 18 by interphase FISH and an unexpected result by array-CGH

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Interphase FISH was performed on a chorionic villi sample (CVS) using the Aneuvysion® panel yielding a positive result for trisomy 18. Karyotyping revealed a contradictory result of 46,XY. Metaphase FISH showed an ectopic 18 cen signal present at 9p12. Additional FISH studies showed that 9 cen- and 9qh-specific probes hybridized in a normal pattern on the two chromosome 9s. This abnormal pattern was also observed in the phenotypically normal mother of this fetus, demonstrating maternal inheritance. Array-CGH was performed on DNA extracted from chorionic villi and showed no significant deletions or duplications on chromosome

9. However, the array result serendipitously diagnosed an unrelated clinically significant deletion in the Miller-Dieker syndrome (MDS) critical region. A similar false positive trisomy 18 due to an apparent benign variant on chromosome 9 has previously been reported by Wei et. al. (Prenat Diagn: 27(11), 2007). Laboratory protocol has since been modified to include the precautionary measure of performing an additional reflex test on all positive trisomy 18 cases.

Preliminary examination of long contiguous stretches of homozygosity in individuals from isolated communities compared to outbred or consanguineous populations

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SNP microarrays are becoming increasingly important in the genetic investigation of individuals with various phenotypes. In addition to pathogenic CNVs, they also enable detection of long contiguous stretches of homozygosity (LCSH) that may be associated with autosomal recessive or imprinted loci. LCSH patterns have been examined in various outbred populations yet the frequency, average length and significance of these loci in isolated populations remains poorly understood. In order to better understand the LCSH patterns in isolated populations compared to outbred or consanguineous populations 62 consecutive cases were reviewed that had been referred for microarray testing using the Affymetrix SNP6.0 platform. These included five individuals from isolated communities in either Nunavut or Northern Quebec and nine individuals from consanguineous matings. The individuals from the isolated communities showed fewer number of LCSH than those from consanguineous matings (average 2.0 versus 7.5 LCSH >10 Mb, respectively), but more than observed in the outbred individuals (average 0.08 LCSH >10Mb). The average identity by descent (>10 Mb) was calculated to be ~1% of the autosomal genome among individuals from the isolated communities. Again this is lower than expected given a history of consanguinity, but higher than expected for outbred populations. These preliminary findings support the hypothesis that individuals from isolated communities are likely to share more regions of autozygosity than outbred populations but less than observed from consanguineous matings; better characterization of the LCSH patterns from isolated communities has the potential to identify loci that contribute to phenotypes within these communities and warrants further investigation.

Moving from chromosome analysis of cultured amniocytes: implementation of SOGC guidelines for amniotic fluids in the Champlain LHIN

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Over the last several years many diagnostic labs have introduced QF-PCR as a rapid prenatal screen for aneuploidy involving chromosomes 13, 18, 21, X or Y but have maintained the requirement to follow-up all QF-PCR tests with full chromosome analysis of cultured amniocytes. With increased experience and confidence in QF-PCR as a stand-alone test, along with the availability of prenatal microarray, there is acceptance of the idea that QF-PCR can replace conventional cytogenetic analysis whenever prenatal testing is performed solely because of an increased risk of aneuploidy for chromosomes 13, 18, 21, X or Y. In Sept 2011, the

Society of Obstetricians and Gynaecologists of Canada (SOGC) and the Canadian College of Medical Geneticists (CCMG) published a joint clinical practice guideline entitled "Use of a DNA Method, QF-PCR, in the Prenatal Diagnosis of Fetal Aneuploidies". Implementing this major change in analysis criteria concurrent with increased availability of prenatal microarray brings its own set of challenges: it is highly desirable to maintain a DNA source for all pregnancies with normal QF-PCR results. For more complex cases with US abnormalities there is the need to have samples available for possible microarray testing; trisomy-only risk cases may have anomalies detected later in the pregnancy necessitating microarray. We have developed an algorithm for prenatal testing for trisomy-risk pregnancies vs those at risk for other genetic imbalances which allows for the maintenance of a DNA source without extensive culturing. Our approach reduces chromosome analysis of cultured amniocytes by 75% while maintaining clinically appropriate testing material.

Comparison of nuclear organization in motile and immotile human spermatozoa analyzed by Fluorescence in Situ Hybridization (FISH) with 3D image analysis

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Objective: Human spermatozoa have unique, well-organized nuclear architecture different from somatic cells. Studies have shown that chromosomes in human spermatozoa are arranged non-randomly with centromeres of non-homologous chromosomes forming a chromocenter. The purpose of our study was to evaluate structural difference between motile and immotile human spermatozoa using 3D digital image analysis.
Methods: The study was performed on three normozoospermic patient ejaculates. Each sample was divided into two subgroups of motile and immotile sperm using gradient density centrifugation. The chromocenter and centromere of chromosome 17 was localized using FISH and 3D imaging using deconvolution technique and analyzed by IMARIS software.
Results: Significant differences were observed between motile and immotile spermatozoa's volume of nucleus, centromere of chromosome 17 and volume of chromocenter. The immotile sperm population had significantly higher disrupted structure of chromocenter and localization of chromosome 17 compared to motile sperm. "Ideal" cells were defined as one with 1 to 3 chromocenters and preferred radial and longitudinal position of chromosome 17 which were observed in 52% of motile spermatozoa in comparison to 32% of immotile population,($p < 0.05$).
Conclusions: The motile sperm population has a higher number of cells with properly organized nuclear structures when compared to immotile population. 3D image analysis allows advance understanding of sperm spatial nuclear organization, in spite being labor intense. This data might provide additional information on exceptionally structured sperm chromatin involved in the formation of normal sperm chromosome organization, paramount at the time of fertilization and embryo development.

Array CGH – Coming of age in the diagnostic laboratory

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Cytogenetics has been a cornerstone of genetic diagnostic testing, playing an important role in elucidating the underlying causes of congenital anomalies, developmental disorders, infertility and cancer. Despite forecasts of its imminent demise, basic karyotype analysis has changed very little over the past 40 years. The advent of molecular karyotyping in the form of microarray however, has now assumed a role of primary importance in the diagnostic laboratory setting, revealing a new layer of genomic changes responsible for human diseases in the submicroscopic copy number differences.

The Alberta Children's Hospital Cytogenetics Laboratory has now conducted over 1000 array CGH analyses for suspected constitutional genomic imbalances. As we introduced this technology in the clinical cytogenetic laboratory, there have been challenges, surprises and rewards. Illustrative examples of each will be discussed.

Targeted mutation analysis in oncology: personalized medicine at work

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Next generation sequencing (NGS) technology is revolutionizing cancer research with unprecedented amounts of genome-wide data being generated for many tumor types. This is leading to a greater recognition of many of the molecular events associated with cancer initiation, progression and response to treatment. Data will be presented that compare the use of NGS technology and high throughput genotyping in a routine clinical lab and then assess the feasibility of its use in a multi-center clinical trial setting. The objectives of the study were to determine whether it is possible to analyze patient samples using NGS and MassArray genotyping within a timeframe that is clinically useful and to optimize processes and procedures for genomic analyses (somatic mutation genotyping and targeted exome sequencing) of 'current' tissue samples from cancer patients. Feasibility for use of this technology was defined as: greater than 50% of patients approached being recruited, acceptable biopsy samples in $\geq$90% of cases, successful analysis: $\geq$90% of cases, time to result $\leq$21 days in $\geq$90% of cases and $\geq$30% of mutations found being actionable. Advantages and limitations of the genotyping technology will be discussed, as well as its utility for targeted therapy of solid tumors and its current use in a clinical trial at PMH.

Relating centromere topography in fixed human chromosomes to a-satellite DNA and CENP-B distribution

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We have previously shown allelic differences in accessibility to a subset of short (1500-5000 basepairs) fluorescence *in situ* hybridization (FISH) DNA probes on metaphase chromosomes. To begin to examine such differences, the topography of metaphase chromosomes visualized by atomic force microscopy (AFM) was correlated with the organization of integral chromosomal proteins by immuno-FISH. Initially, a-satellite DNA on centromere 17 marked by CEN-17 FISH

was correlated with chromosome topography. The distribution of a-satellite DNA fluorescence was not concordant with the centromere topography in 70% of the homologs examined. AFM also revealed prominent ridge-like structures along the lateral axis of centromere 17, with an average ridge width of 0.74 µm. Immuno-localization of CENP-B, a constitutive protein associated with a-satellite DNA, coincided with the centromere ridge topography. Additional immuno-FISH studies showed that in ~ 50% of cells, the distributions of interacting CENP-B protein and a-satellite DNA was not consistent during metaphase. Variability in chromosome topography was also studied at non-centromeric regions. Two distinct short low copy sequence-defined FISH probes spanning 3.4 Kb, and detecting separate homologous targets within *NOMO3* and *PM5* on chromosome 16p, were analyzed by AFM. Generally, these probes more frequently hybridized to groove-like topographic features (n = 27) as compared to ridge-like features (n = 8) on metaphase chromosomes. The preference for short DNA probe (3.4 Kb) hybridizations relative to the observed topographic features may be related to the chromatin compaction state or the distribution of underlying epigenetic marks in mitosis.

Preimplantation genetic screening: The Mount Sinai experience

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Preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS) refer to procedures used to analyze embryos prior to implantation, improving the chance of conception for patients that are either at high risk of transfering specific inherited disorders (Zheng *et al*, 2011) or those of advanced maternal age. For the last twenty years, PGD has been mostly performed on cells from cleavage stage embryos using polymerase chain reaction (PCR) to detect monogenic disorders or fluorescence in situ hybridization (FISH) to analyze chromosomes (Harper *et al*, 2010; Harper *et al*, 2012). With the introduction of the 24sure(+) aCGH platforms, BlueGnome, Ltd, has provided a more efficient and informative approach to both PGS for aneuploidy detection, and PGD of inherited unbalanced translocations.
A single cell (ie. polar body, blastomere, or blastocyst) corresponding to it's respective oocyte or embryo, is amplified (whole-genome amplification), labelled and hybridized to a bacterial artificial chromosome (BAC) microarray, providing 10Mb coverage across all chromosomes. Results are obtained in as little as 12 hours, allowing infertility (IVF) clinics to provide fresh (next-day) transfers of embryos without the need for criopreservation of the blastocyst(s). With the establishment of a microarray facility in 2010, Mount Sinai Hospital has been providing both PGD and PGS to infertility clinics in the Greater Toronto Area.

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Next generation genomic microarrays and custom FISH probes for molecular cytogenetic analysis designed by *ab initio* sequence analysis

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Array comparative genomic hybridization (aCGH) and fluorescence *in-situ* hybridization (FISH) are recommended as first-line tests for detection of chromosomal abnormalities in patients with congenital disorders and malignancies. Current aCGH platforms exhibit high coefficients of variation and low reproducibility in raw intensities, due to the use of blocking DNA, which is contaminated with single copy sequences. To improve the performance and define the content of FISH probes and microarrays, we have implemented an *ab initio* method (US Pat No 7,734,424), which locates unique genomic sequence intervals without repeat-masking. Our 44K *ab initio* aCGH microarray has lower coefficients of variation relative to Agilent's 4x44K platform ($p \leq 0.001$). *Ab initio* probe sequences were able to detect a previously mapped Angelman Syndrome deletion (15q11.2-q13) in all replicates, compared to Agilent probe sequences, which called the deletion in one of three replicate arrays. Short single copy (scFISH) probes based on *ab initio* designs improve detection of subtle disease-causing abnormalities for genes not covered or too small to be accurately detected by recombinant BAC probes. Chromosomal hybridization at high stringency produces the expected metaphase hybridization pattern. scFISH probes have been synthesized and validated for *CDKN2A*, *TP53*, *CCND1*, *ERBB2* and *NOTCH1.* A genome-wide set of scFISH probes has been designed, covering approximately 98% of all *ab initio* single copy intervals and all known unique genes. These scFISH probes characterize chromosomal abnormalities in tumours at a resolution equivalent to Southern blot analysis and can be used to validate small acquired rearrangements seen by aCGH.

Rapid aneuploidy screening by QF-PCR and the changing scene in prenatal diagnosis

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Chromosome analysis of cultured cells has been considered the standard method for prenatal diagnosis since its clinical implementation in the early 1970s. The main limitation remains the need of cell culture, resulting in a reporting time of ~14 days. Improvements in non-invasive screening methods for chromosome abnormalities have radically changed the indications for prenatal diagnosis over the last decade. This prompted the introduction of molecular methods to detect common aneuploidies in a few hours from sampling.

Rapid prenatal diagnoses of common aneuploidies have successfully been achieved by Quantitative Fluorescent PCR (QF-PCR) amplification of selected microsatellite. The analyses of large series of clinical samples have shown that this approach has a very high rate of success and, although being deliberately targeted to the analysis of selected chromosomes, it can detect the great majority of chromosome abnormalities in prenatal diagnosis. Retrospective studies have also suggested that abnormal fetal ultrasound can be considered as main indication to decide if QF-PCR should be followed by fetal karyotype. This strategy has been adopted with different approaches by several centres in different countries.

More recently, the advent of microarray based comparative genomic hybridization (aCGH) has significantly increased the sensitivity to detect copy-number variants. With its commercially available platforms, these methods allow rapid whole genome scanning and identification of submicroscopic imbalances, most of which are actually not object of prenatal screening. While

its increasing application on prenatal samples from fetuses with abnormal ultrasound improves pregnancy management, it is also modifying the indications for invasive diagnostic procedures. We are going through a period of transition where improved non invasive screenings based on biochemistry, ultrasound, eventually also including free fetal DNA analysis, will further decrease the number of invasive prenatal diagnostic procedures.
However, in high risk pregnancies, rapid confirmation of screening results will always be required and QF-PCR should be considered as the elective choice to exclude the presence of common aneuploidies before aCGH analysis or fetal karyotyping.

Automation of array CGH processing

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Genetic Laboratory Services, AHS

Array Comparative Genome Hybridization (aCGH) has been able to provide Cytogeneticists a valuable tool to resolve genomic imbalances with higher resolution and details. For these reasons, aCGH has been on the rise within the last several years in labs across Canada. The benefits of this technology are quite evident; however, the process of preparing patient samples can be very labour intensive. Steps can be automated at several stages to increase efficiency: accessioning, pre/post sample labeling, pre/post hybridization, data analysis, aberration validation, and report generation. We will give an overview of our lab's experience automating part of the processing steps involved in aCGH work flow and the benefits we have gained.

The Philadelphia Chromosome Story

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How the discovery of a unique chromosome abnormality in chronic myeloid leukemia (CML) morphed/evolved into remarkably effective therapy roads like a scientific fairy tale and highlights the role of good luck, perseverance and collaboration in successful scientific research.

It began in 1960 with the discovery by David Hungerford collaborating with Peter Nowell that CML cells have one small chromosome that is very small. It was called the Philadelphia or Ph Chromosome. With banding each chromosome could be distinguished. I showed in 1972 that the Ph Chromosome was not the result of a deletion but was involved in a translocation with chromosome 9. How the translocation was related to leukemia was completely unknown.

Other scientists especially David Baltimore and Fellows in his laboratory showed that the Abelson virus could transform fibroblasts and lymphocytes which could then form tumors in mice. Others showed that the human *ABL* gene, normally on chromosome 9, was on the Ph chromosome. John Groffen and Nora Heisterkamp cloned the translocation breakpoint and showed that it represented a chimeric fusion gene of *BCR* on chromosome 22 and *ABL*. Because *ABL* was a tyrosine kinase, and pharmaceutical companies were developing kinase inhibitors, Brian Druker working with Nick Lydon screened a number of these compounds and found one

that inhibited the growth of CML cells. Thus Gleevec®/imatinib was "born" and it has revolutionized treatment of CML.