

Clonal Evolution Predicts Poor Prognosis in Chronic Lymphocytic Leukaemia and Is As Significant A Marker of Poor Prognosis as High Risk Cytogenetic Markers 17p- and 11q-: A Population Based Cohort study of FISH Testing in British Columbia, Canada

Adam C. Smith¹, Krystal Bergin², Alina S. Gerrie², Steven J.T. Huang³, Helene Bruyere³, Chinmay B. Dalal², Khaled M. Ramadan⁴, Monica Hrynychak⁵, Aly Karsan⁶, Cynthia L. Toze², Tanya L. Gillan³.

¹Instituto de Pesquisa Pelé Pequeno Príncipe, Curitiba, Brazil

²British Columbia Cancer Agency, Vancouver, Canada

³Vancouver General Hospital, Vancouver, Canada

⁴Division of Hematology, St. Paul's Hospital, Vancouver, Canada

⁵Royal Columbian Hospital, New Westminster, Canada

⁶BC Genome Sciences Centre, Vancouver Canada

Chronic lymphocytic leukaemia (CLL) is the most common form of leukaemia in North America. Chronic lymphocytic leukemia (CLL) was originally reported to be a karyotypically stable disease in the first serial studies performed by karyotyping; however, later studies have demonstrated that clonal evolution occurs in CLL and is visible by karyotyping. The development of FISH testing has also allowed for the interrogation of chromosomal alterations that are not easily visible by metaphase cytogenetics, such as deletion of 13q or deletion of TP53 (del17p). The British Columbia CLL database contains clinical and molecular cytogenetic data on more than 900 patients. Patients that had multiple FISH analyses performed (n=134) and were analyzed for the presence of clonal evolution detected by FISH (n=23). Patients with clonal evolution had a worse outcome compared to the non-clonal evolution group with a median overall survival (OS) from diagnosis of 10.3 years compared with 19 years, hazard ratio (HR) 5.9 (95% CI 2.1, 13.7, p=0.001). This effect maintains its significance on multivariate analysis after adjustment for sex, age, Rai stage, lymphocyte count at diagnosis and baseline cytogenetic abnormalities after adjusting for timing of FISH testing: HR 3.24 (95% CI 1.23-8.55, p=0.018). When considered from date of 1st FISH clonal evolution continues to retain its poorer prognosis with an OS of 5.1 years versus not reached (non-CE, n=111) p= 0.033. The only other variable associated with a significantly shorter OS was the presence of 17p- on initial FISH test: HR 3.60, 95% CI 1.16-11.18, p=0.027. Interestingly, the presence of clonal evolution during the course of the disease appears to be as negative a prognostic factor as the presence of del(17p) at initial diagnosis making the detection of clonal evolution an extremely important clinical variable.

Scavenger hunt & disappearing act in a pediatric pre-B ALL: Why Haematopathologists are Cytogeneticists' best friends

M Beaulieu Bergeron^{1,2}, J McGowan-Jordan^{1,2}

¹Children's Hospital of Eastern Ontario & Ottawa; ²Department of Pathology and Laboratory Medicine, University of Ottawa.

We present here the case of a 7 year-old male diagnosed with pre-B ALL in whom standard chromosome analysis showed the following karyotype: 52,XY,+X,+Y,+14,+21,+21,inc[4]/52,XY,+X,+Y,+14,+21,+21,+?mar,inc[4]/46,XY[3]. While high hyperdiploidy (51-65 chromosomes) with double trisomy 4 and 10 is very frequent and of favourable prognosis in pediatric pre-B ALL, "hyperdiploidy by tetrasomy" of chromosomes 14, 21 and X/Y has been associated with the presence of a masked near-haploid clone and a poor prognosis. Interphase FISH analysis with probes for BCR-ABL, ETV6-RUNX1, CEP4&10, MLL and IGH did not detect any of the most common structural rearrangements in pediatric pre-B ALL, nor did it detect the presence of a near-haploid clone. The remains of the EDTA blood samples drawn at diagnosis for

flow, CBC and TM, was used for SNP array analysis which confirmed the observed gains of chromosomes X, Y, 14 and 21 but also demonstrated a marked skewing of the alleles on the other chromosomes. These results were consistent with duplication of a single homologue for all chromosomes except X, Y, 14 and 21 in the abnormal leukemic cells due to endoreduplication of a masked near-haploid clone. We are now in the process of changing our procedure so that EDTA samples drawn at diagnosis for CBC or TM are sent for DNA extraction, rather than being discarded, for all new pediatric leukemic patients in order to allow further analysis by SNP array if required.

Integrating mutation panel testing and copy number in clinical genomic studies

Joshua Schiffman, MD
University of Utah

Cancer strikes 50% of all men and 33% of all women through their lifetime. Relapse is the leading cause of cancer-related death due to ineffective therapies because of tumor heterogeneity and molecular evolution. New technologies, such as genome-wide copy number analysis and mutation analysis, enable researchers to identify clinically actionable targets for improved treatment outcome. We enrolled patients with relapsed solid tumors at Huntsman Cancer Institute at the University of Utah and profiled their tumors for actionable mutations (Sequenom, N=202), including a subset that were analyzed for copy number changes (OncoScan, Affymetrix, N=48). The results were integrated to determine the total number of patients that contained actionable items in each tumor subtype. This talk will describe this trial in more detail, and will discuss the rationale use and trial design for clinical genomics.

Whole-genome scanning by SNP array reveals novel chromosome aberrations in chronic lymphocytic leukemia - a comparison study of CLL FISH and Affymetrix CytoScan®

Hong Wang and Kathy Chun
Genetics Program, North York General Hospital, Toronto, Canada

Chronic lymphocytic leukemia (CLL) is a highly heterogeneous disease with a wide range of genetic aberrations. The genetic heterogeneity of CLL requires sensitive molecular cytogenetic tools that can detect the genomic changes in a small proportion of malignant cells. However, conventional G-banding analysis of CLL is limited due to the low mitotic activity of CLL cells. FISH analysis is also restricted to the genetic aberrations defined by the applied probe panel. Therefore, we hypothesized that whole genome analysis using SNP array would be useful to detect the diversity of genomic imbalances as well as copy-neutral loss of heterozygosity (cnLOH) in CLL. In this study, we performed SNP array analysis using the Affymetrix CytoScan® HD array on 50 patients diagnosed with CLL, in comparison with FISH analysis. SNP array detected nearly all abnormalities that were identified by FISH, including monoallelic and biallelic deletions of 13q14, deletion of ATM, trisomy 12 and deletion of TP53. The results of SNP array and FISH analyses were 98% in concordance. In addition to the aberrations defined by the FISH probe panel, SNP array revealed other genomic aberrations, including cnLOH (also known as acquired uniparental disomy) and chromothripsis. Additional recurrent copy number gains and losses and trisomies were also

detected. Moreover, SNP array analysis demonstrated a huge heterogeneity of genomic complexity regarding the size and number of copy number alterations among CLL patients. In conclusion, SNP array analysis provides a comprehensive tool for delineation of genetic changes in CLL with high resolution.

The *ZNF384* gene in pediatric leukemia – multiple partner genes, immature (CD10 negative) immunophenotype, and potential good outcome

Mary Shago^{1,3}, Oussama Abba^{2,4}, Johann Hitzler^{2,4}, Sheila Weitzman^{2,4} and Mohamed Abdelhaleem^{1,3}

Departments of Paediatric Laboratory Medicine¹ and Paediatrics², The Hospital for Sick Children, Departments of Laboratory Medicine and Pathobiology³ and Pediatrics⁴, University of Toronto.

The *ZNF384* (*CIZ*) gene, located distal to the *TEL* (*ETV6*) gene at 12p13.31, is a putative zinc finger transcription factor which has been reported to be rearranged in acute leukemia. Rearrangement of the *ZNF384* gene results in attachment of various 5' partner gene sequences to form *ZNF384* fusion genes. The *ZNF384* gene has three known partners: *TAF15* at 17q12 (19 cases), *EWSR1* at 22q12 (4 cases), and *TCF3* (*E2A*) at 19p13 (3 cases). The proteins encoded by the fusion genes consist of the transactivation domains of the 5' partner genes fused to the entire coding region of *ZNF384*. I will present details of seven pediatric pre-B ALL patients with *ZNF384* gene rearrangement. Our patients had lymphoblasts with a CD10-negative immunophenotype, similar to the immunophenotypic profile seen in *MLL* gene-rearranged ALLs. Follow up on the patients ranges from 6 to 8 years, and none of the patients have relapsed. Identification of the rearrangements was facilitated using dual-colour breakapart probes for the *ZNF384*, *TCF3*, and *EWSR1* loci. Four of the patients had *TCF3-ZNF384* gene rearrangement and one had *EWSR1-ZNF384* gene rearrangement. Two patients had novel *ZNF384* gene translocations involving regions on chromosomes 6 and 22, identified as the *ARID1B* and *EP300* genes respectively by RNA sequencing analysis. Similar to the previously identified *ZNF384* partners, these novel partner genes, which function in chromatin remodeling, contribute 5' gene sequences to the *ZNF384* gene fusion. Based on the total number of ALL patients seen at our centre during the study period, our data suggests that, collectively, *ZNF384* gene rearrangement may have an incidence of ~3% in pediatric pre-B ALL, with an incidence of at least 18% in CD10-negative pre-B ALL. *ZNF384* gene rearrangement may be associated with a more favorable prognosis than *MLL* gene rearrangement. Both good and poor outcomes have been proposed in the literature, however, the majority of the data are based on *TAF15-ZNF384* rearrangement in adult patients. The *ZNF384* gene rearrangements described above are cryptic and not easily identified by *G*-banding. Performance of *ZNF384* FISH analysis in patients with CD10-negative ALL, after *MLL* gene rearrangement has been excluded, will assist with the determination of the prognostic significance of *ZNF384* gene rearrangement in pediatric ALL.

Developing RNA and DNA profiling to identify leukemia patients most likely to benefit from targeted therapeutics

Jean McGowan-Jordan^{1,2,3}, Kristin D. Kernohan^{1,2}, Sarah L. Sawyer^{1,2}

¹Department of Genetics, Children's Hospital of Eastern Ontario, University of Ottawa, Ottawa, ON, Canada

²Children's Hospital of Eastern Ontario Research Institute, University of Ottawa, Ottawa, ON, Canada
Ontario, University of Ottawa, Ottawa, ON

³Department of Pathology and Laboratory Medicine, University of Ottawa, Ottawa, ON

Pre-B acute lymphoblastic leukemia is the most common childhood cancer and is caused by acquired genetic mutations and rearrangements. Tyrosine kinase inhibitors (TKIs) have improved survival from 30% to 80% in pre-B ALL patients with t(9;22), a well characterized rearrangement which creates the constitutively active tyrosine kinase, BCR-ABL1. New research suggests TKIs may also be effective in other Pre-B ALL subtypes which exhibit similar gene expression and mutation patterns. Advances in treatments based on molecular profiles imply a responsibility to correctly and efficiently identify all patients who will benefit, and those who will not. To do this, we have developed targeted RNA and DNA next generation sequencing panels to analyze ~300 transcripts from pre-B ALL bone marrow to develop an algorithm for the identification of patients with BCR-ABL1-like leukemias, who are likely to benefit from targeted treatment. Molecular profiling in pediatric oncology patients has the potential to minimize adverse reactions to medications, tailor treatments to individual patients, improve outcomes, and more effectively manage health care resources. To our knowledge, this BCR-ABL1-like identification protocol will be the first in Canada, and sets a precedent for the inclusion of transcriptional profiles more broadly in genetic diagnoses and identification of pathways that can be targeted for therapeutic translation.

***NRXN1* Gene Deletions Dissected**

Marsha D. Speevak, Department of Genetics and Laboratory Medicine, Trillium Health Partners

NRXN1 is a large, dosage sensitive gene that encodes multiple isoforms of Neurexin-1 using two different promoters. The upstream promoter produces the majority of transcripts known as alpha-neurexin isoforms. The downstream promoter produces the beta-neurexin isoforms which are much shorter and less plentiful than alpha-neurexins. The neurexins are exclusively expressed in the neurons as neuronal cell surface receptors. Deletions in *NRXN1* at 2p16.3 are associated with several different phenotypes including developmental delay, dysmorphism, autism, epilepsy and schizophrenia. Deletions may be inherited, or *de novo*, leading to the supposition that *NRXN1* abnormalities are incompletely penetrant and variably expressed.

Due to the reports of *NRXN1* CNVs in control populations (DGV database), distinguishing likely causative *NRXN1* CNVs from background is particularly problematic. To address this, the distribution, exonic content and inheritance patterns of *NRXN1* CNVs were examined in an Ontario postnatal patient population. Out of approximately 6400 cases, a total of 57 *NRXN1* CNVs were detected representing a 0.89% detection rate. The vast majority of these were deletions (54/57). Not all were reported due to failure to meet minimum reporting criteria (very small and/or intronic). Most intronic CNVs clustered in introns 1 and 2 of either isoform, and several had recurrent breakpoints (6.6Kb, 11Kb; 24Kb and 71Kb). There was a high rate of unrelated reportable CNVs among the intronic variants in this data set (15/34). Of these, eight CNVs could be categorized as clearly pathogenic/likely pathogenic and the rest were considered VUS. Among the 23 exonic CNVs only five had additional reportable CNVs of which one (a 15q BP3-BP4 deletion) was considered potentially contributory. The exonic deletions tended to include exon 1, exon 2 or both exon 1 and exon 2 of the alpha isoform of *NRXN1*. Follow-up was obtained for 16 families with the following breakdown: 6 *de novo*; 7 maternal and 3 paternal. The smallest *de novo* deletion was 88.5Kb in a one year old with global delay and the largest was approximately 800Kb in an 11 year old child with developmental delay. The largest inherited deletion found so far was a paternal origin deletion of 275Kb in a young adult male with ADHD and autism.

The distribution of *NRXN1* CNVs among patients is very similar to that seen in control populations. However, from these data, it may be concluded that the small, recurrent, intronic CNVs are unlikely to carry any clinical significance and may be safely suppressed. Location (alpha-isoform), content (exonic) as well as uniqueness (particularly towards the 5' end) of the CNV raises the probability that it contributed to the phenotype. These findings may prove helpful when reporting and/or counselling, particularly in the setting of prenatal diagnosis.

Group presentation on interesting cases with unusual or challenging observations

Rosemary Mueller

Victoria General Hospital, Victoria BC

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

Karyotyping is here to stay	Hong Wang	North York General Hospital
Case Report: Chromosome Instability in a neonate, True or Artifact?	Marsha Speevak	Credit Valley Hospital
An interesting case of an interstitial deletion and ring chromosome.	Entela Ketri	Mount Sinai Hospital
Metastatic rhabdomyosarcoma mimicking acute leukemia in a pediatric patient	Shanthini Kangesan	The Hospital for Sick Children
Detection of an 8Mb 14q32 microdeletion: a case report illustrating the importance of highlighting cancer susceptibility genes in the microarray report	Ted Young	The Hospital for Sick Children
Of Wood's lamp and Blaschko lines: When it pays off to look at the skin	Melanie Beaulieu Bergeron	Children's Hospital of Eastern Ontario
A complex inv dup/del in chromosome 2q	Judy Chernos	Alberta Children's Hospital

Four deletions and 9 breaks resulting from a rearrangement between 2 chromosomes, as observed in a patient referred for short stature and global developmental delay.

Fléchère Fortin¹ and Sébastien Chénier¹.

¹ Clinical cytogenetics laboratory, Sherbrooke University Health Centre; Department of pediatrics, University of Sherbrooke.

A 9-year-old patient was referred in medical genetics for short stature and global developmental delay. Examination revealed the presence of minor dysmorphies, epilepsy, moderate mental retardation and dysphagia. Array CGH analysis shows that 4 interstitial deletions are present: in 4q13.1q13.2, in 4q13.2q13.3, in 5p14.1p14.3 and in 5q34q34. The karyotype reveals interesting surprises: an insertion (4;5) coupled with a paracentric inversion (5). All cytogenetic results are

shown (aCGH, Karyotype, FISH, m-BAND FISH), and constitutional chromotripsis is briefly presented as a potential cause for this complex rearrangement involving at least 9 breaks.

Array CGH and molecular cytogenetics characterization of Pallister-Killian Syndrome

Judy Chernos¹, Bob Argiropoulos¹, Joanna Lazier² and Faezeh Vasheghani³

¹Cytogenetics Laboratory, Alberta Children's Hospital, Calgary, Alberta. ²Clinical Genetics Unit, Alberta Children's Hospital, Calgary, Alberta. ³Department of Human Genetics, University of Paris Descartes-V, 15, Ecole de medicine St., 75006, Paris, France.

Pallister-Killian Syndrome (PKS) is a rare sporadic genetic disorder which is caused by tissue-limited mosaicism of a supernumerary isochromosome 12p or intrachromosomal duplication of the short (p) arm of chromosome 12. We present here three cases with features of PKS that were diagnosed by array CGH to have structurally different cytogenomic imbalances. Further molecular characterization demonstrated that one iso 12p marker was an anaphoid chromosome with a neocentromere. Clinical diagnostic features and appropriate genetic testing for suspected PKS will be discussed.

A brief history of cell and tissue culture

AnneMarie Block, PhD, FACMG

Department of Pathology and Laboratory Medicine, Roswell Park Cancer Institute

Roswell Park Memorial Institute was at the forefront of development of tissue culture media and cell line establishment in the 1960s. Anecdotes from the early days of tissue culture, researchers who helped develop the techniques used today and the products they created will be discussed.

Complex structural chromosomal rearrangements involving 8p23: a rare case of 8p23.1 duplication and 8p23.2 triplication

Kathleen Bone, Bob Argiropoulos and Judy Chernos

Cytogenetics Laboratory, Alberta Children's Hospital, Calgary, Alberta

Recurrent chromosomal rearrangements in 8p23.1, including inverted duplication deletion 8p (inv dup del8(p23)), 8p23.1 Deletion Syndrome, 8p23.1 Duplication Syndrome, and the translocation t(4;8)(p16;p23), are mediated by non-allelic homologous recombination (NAHR) at two complex low copy repeats (LCRs). An inversion polymorphism present in approximately 25% of the population causes susceptibility to NAHR between the LCRs in this region, leading to the formation of these aforementioned rearrangements that carry some degree of structural complexity. Here, we report on a rare and complex recurrent rearrangement consisting of a 4.94 Mb 8p23.1 duplication and a 4.67 Mb 8p23.2 triplication in a 7 year old male with developmental delay and autism. The genomic imbalance of this patient was detected by array comparative genomic hybridization (aCGH) and confirmed by karyotype. This duplication and triplication of 8p (dup trp 8p23) has been previously reported in two patients with phenotypes distinct from the better characterized inv dup 8p Syndrome, and with variable clinical presentation. The consequences of the genomic architecture of

chromosome 8 will be presented, including suggested mechanisms leading to more complex chromosomal rearrangements involving NAHR at multiple LCRs along the length of the chromosome.

ISCN: How it's made...

Jean McGowan-Jordan^{1,2,3}

¹Department of Genetics, Children's Hospital of Eastern Ontario, ²Children's Hospital of Eastern Ontario Research Institute,

³Department of Pathology and Laboratory Medicine, University of Ottawa

The International System of Human Cytogenetic Nomenclature (ISCN) is a widely used tool and reference which enables chromosome abnormalities to be described in a coherent and uniform fashion, facilitating communication not only between cytogeneticists, but between cytogeneticists and other physicians and scientists. Over the past several decades the ISCN has been assembled, modified, and edited by a group of cytogeneticists, known as the ISCN Standing Committee, with input from the cytogenetics community at large. The most recently discussed upcoming changes to the ISCN will be reviewed, some of which highlight the unique challenges and opportunities for the cytogenetics community resulting from advances in genomic technology.

Localized, structural differences in condensation of homologous metaphase chromosomes and the underlying mechanism.

Joan H.M. Knoll^{1,3,4}, Wahab A. Khan^{1,3} and Peter K. Rogan^{2,3,4}

¹Department of Pathology and Laboratory Medicine; ²Departments of Biochemistry and Computer Science; ³University of Western Ontario, London, ON; ⁴Cytognomix Inc, London, Ontario

How do metaphase chromosomes condense? Condensation differences along the lengths of homologous chromosomes are well known but non-polymorphic differences between homologs have not been appreciated. We recently demonstrated localized differences in chromatin accessibility on homologous metaphase chromosomes in ~10% of single copy loci at 1.5 - 5 Kb resolution (Mol. Cytogenet. 7:70, 2014). These differences (referred to as differential accessibility or DA) occur normally in cells and are detected by FISH with short single copy probes. DA is non-random, heritable, reproducible, and locus specific but not unique to specific chromosomes or imprinted regions. By 3D-superresolution microscopy, we demonstrated that hybridized single copy (SC) probe volume and depth have more than a 3 fold difference between accessible and less accessible homologous regions. Subsequently, we employed chromatin modifying reagents to characterize the underlying causes of DA. Inhibition of topoisomerase II α -DNA cleavage complex reversed DA by equalizing DNA superhelicity. DA was not affected by chromosome treatments targeting histone modifying enzymes, cytosine methylation and defects in chromatid cohesion, all of which are also known to cause decondensation. These data suggest DA is a reflection of allelic differences in metaphase compaction dictated by the local catenation state of the chromosome rather than by other epigenetic marks. Allelic differences in chromosome accessibility represent a stable chromatin mark on mitotic metaphase cells. Since many histone marks are erased before or at the entry into mitosis, this has potential implications for the preservation of cellular phenotypes that enable differences in epigenetic marks to be re-established in progeny cells.

Diagnostic Utility of Whole Genome Sequencing as an alternative to chromosomal microarray analysis in Pediatric Medicine

C.R. Marshall¹, D.J. Stavropoulos¹, R. Jobling², D. Merico³, S. Bowdin², N. Monfared⁴, M.S. Meyn^{2,5}, M. Girdea⁵, M. Szego⁶, R. Zlotnik-Shaul⁷, B. Thiruvahindrapuram³, G. Pellicchia³, T. Nalpathamkalam³, M. Brudno^{5,8}, C. Shuman², R. Hayeems⁹, C. Carew⁴, R. Erickson¹⁰, R.A. Leach¹⁰, P. Ray¹, R.D. Cohn^{2,4,5}, S.W. Scherer^{3,4,5,11}

¹Genome Diagnostics, Department of Paediatric Laboratory Medicine, The Hospital for Sick Children, Toronto, ON, Canada;

²Division of Clinical and Metabolic Genetics, The Hospital for Sick Children, Toronto, ON, Canada;

³The Centre for Applied Genomics, The Hospital for Sick Children, Toronto, ON, Canada;

⁴Centre for Genetic Medicine, The Hospital for Sick Children, Toronto, ON, Canada;

⁵Program in Genetics and Genome Biology, Hospital for Sick Children, Toronto, Ontario, Canada;

⁶Joint Centre for Bioethics, University of Toronto, Toronto, ON, Canada;

⁷Department of Bioethics, The Hospital for Sick Children, Toronto, ON, Canada

⁸Department of Computer Science, University of Toronto, Toronto, ON, Canada;

⁹Child Health Evaluative Sciences, The Hospital for Sick Children, Toronto, ON, Canada;

¹⁰Complete Genomics Inc, Mountain View, CA, USA

¹¹Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada.

Chromosome microarray analysis (CMA) is the current standard as a first tier genetic test for those individuals presenting with developmental delay and/or congenital abnormalities. However, up to 90% of patients who undergo CMA do not obtain a genetic diagnosis leaving physicians to pursue other targeted molecular genetic testing. Whole genome sequencing (WGS) promises to capture all classes of genetic variation in a single experiment but the diagnostic yield of WGS compared to CMA in the clinical setting has not been established. We performed WGS on 100 consecutive patients referred to a pediatric genetics service with clinical indication(s) for CMA. In 31% of cases, WGS identified variants meeting clinical diagnostic criteria representing a 4-fold increase in diagnostic rate over CMA (8%) alone and >2-fold increase in CMA plus targeted testing (15%). WGS identified all rare reportable CNVs that were detected by CMA including *de novo* pathogenic CNVs affecting 4p16.3 and 22q11.2 associated with Wolf-Hirschhorn and 22q11.2 microdeletion syndromes, respectively. In an additional 23 patients, WGS revealed clinically significant sequence level variants presenting in a dominant (65%; including variants in EP300, GDF5, PIK3R2, PACS1, CCM2, SPTAN1) or a recessive (35%; including variants PANK2, LARP7, TSEN54 and NGLY1) manner. Similar to previous studies we found that 4% of cases had variants in at least two genes involved in distinct genetic disorders, contributing to a more complex clinical phenotype. Clinical implementation of WGS as a single genetic test will provide a higher diagnostic yield than conventional testing while decreasing the number of genetic tests and ultimately the time before reaching a genetic diagnosis.

Next Generation Diagnostics for Personalized Cancer Medicine

Tracy Stockley, PhD, FCCMG, FACMG

Associate Director, Molecular Diagnostics Division, Laboratory Medicine Program, University Health Network

Personalized cancer medicine is the use of genomic information to determine prognosis, treatment and management for oncology patients. This presentation will highlight the emerging use of Next Generation Sequencing technologies in clinical diagnostic genomics for oncology, with a focus on specific clinical research studies at the Princess Margaret Cancer Centre, University Health Network. Studies presented will include solid tumor NGS molecular profiling for genotype-matched clinical drug trials (the IMPACT study) and use of genomic information to determine patient

treatment and management in leukemias (the AGILE study). Future opportunities and challenges for implementation of NGS into clinical diagnostic laboratories will be highlighted.

Genomic DNA Methylation Screening in Patients with Developmental and Intellectual Disabilities

Bekim Sadikovic, PhD, DABMG (Cyto; Mol), FACMG
Associate Professor, Pathology and Lab Medicine, Western University
Clinical Molecular Geneticist, London Health Sciences

DNA methylation defects are associated with both pediatric and adult-onset conditions, with a growing list of disorders requiring clinical DNA methylation testing. While some single-gene tests for disorders associated with specific DNA methylation defects exist, currently, screening of genome-wide DNA methylation is not clinically available. Using the Illumina Infinium HumanMethylation450 BeadChip technology, we aim to develop a clinical database and validate a genome-wide clinical DNA methylation screening test for patients with a wide range of developmental and intellectual disability phenotypes (DD/ID). Here, I present findings from the ongoing multicenter study involving >1000 patients with a broad range of DD/ID phenotypes, genetic and epigenetic conditions. In addition to validating this test for detection of known imprinting/epigenetic disorders, I will present novel types of epigenomic findings in patients with DD/ID that can be grouped in the following categories: a) broad- genome-wide methylation disruption, b) novel imprinting defects, c) methylation defects in OMIM genes with corresponding clinical presentation in patients, e) impact of local genomic alterations on DNA methylation and gene inactivation, and e) identification of diagnostic "Epi-Signatures" associated with gene defects in multiple Mendelian conditions.

Preimplantation genetic screening: An update to the Mount Sinai Hospital Experience

Vlasschaert M, Noor A, Ketri E, Wozniarski A, Foster V, and Kolomietz E.
Department of Pathology and Laboratory Medicine, Division of Diagnostic Medical Genetics, Mount Sinai Hospital, Joseph and Wolf Lebovic Health Complex, Toronto, Ontario

While advances in assisted reproductive technology (ART) has changed the treatment of infertility, implanting embryos with an abnormal number of chromosomes (aneuploidy) is a major cause of IVF failure¹. To combat this issue, chromosome screening can be performed through the use of preimplantation genetic screening and/or preimplantation genetic diagnosis. These technologies refer to procedures used to analyze embryos prior to implantation, improving the chance of conception for IVF patients especially those of advanced maternal age and/or at high risk of transferring an unbalanced form of a chromosomal rearrangement². By selectively implanting euploid embryos, IVF clinics aim to improve IVF success by improving implantation rates, reducing miscarriage rates, all in an attempt to increase ongoing pregnancy rates^{3,4,5}.

Over the last 25 years, preimplantation aneuploidy testing has shifted from FISH to array-CGH and NGS technologies. Biopsy procedures and sample types have moved from polar body to cleavage stage (blastomere) to blastocyst/trophectoderm biopsy. The IVF community has also shifted from

multiple embryo transfers to single embryo transfers, in an effort to decrease the number of high-risk multiple pregnancies.

With the establishment of a microarray facility in 2010, Mount Sinai Hospital has been providing both PGS and PGD to infertility clinics in the Greater Toronto Area.

1. Scott RT Jr, Ferry K, Su J, Tao X, Scott K, et al. (2012) Comprehensive chromosome screening is highly predictive of the reproductive potential of human embryos: a prospective, blinded, nonselection study. *Fertil Steril* 97(4): 870-875.
2. Zheng YM, Wang N, Li L and Jin F. (2011) Whole genome amplification in preimplantation genetic diagnosis. *J Zhejiang Univ Sci B*. 2001 Jan;12(1):1-11.
3. Grifo JA, Hodes-Wertz B, Lee HL, Amperloquio E, Clarke-Williams M, et al. (2013) Single thawed euploid embryo transfer improves IVF pregnancy, miscarriage, and multiple gestation outcomes and has similar implantation rates as egg donation. *J Assist Reprod Genet* 30(2): 259-264.
4. Scott RT Jr, Upham KM, Forman EJ, Hong KH, Scott KL, et al. (2013) Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases in vitro fertilization implantation and delivery rates: a randomized controlled trial. *Fertil Steril* 100(3): 697-703.
5. Forman EJ, Hong KH, Ferry KM, Tao X, Taylor D, et al. (2013) In vitro fertilization with single euploid blastocyst transfer: a randomized controlled trial. *Fertil Steril* 100(1): 100-107.

Molecular subtyping of pediatric tumours using the nanoString technology in a clinical laboratory

Anthony Arnoldo¹, Cynthia Hawkins^{1,2}, and Gino R. Somers^{1,2}

Division of Pathology¹, Department of Paediatric Laboratory Medicine, The Hospital for Sick Children, Department of Laboratory Medicine and Pathobiology², University of Toronto.

Development of new diagnostic strategies capable of delivering accurate and timely information is crucial for the optimization of patient care. NanoString technology has the ability to simultaneously interrogate multiple genetic features that improve the targeted treatment of pediatric diseases, including tumours. The methodology avoids the tedious reverse-transcription or amplification of the target sequence and was validated on various difficult to work with samples (including FFPE tissues and crude cell lysates). As a proof of concept, we utilized the multiplexing capabilities of the nanoString technology to validate the transcript-dependent subgrouping of medulloblastomas. Our validation assays have also demonstrated the efficacy of that approach to accurately classify sarcomas, leukemias as well as to study low-grade gliomas based on the direct detection of distinct fusion transcripts.

Control Fragment Match (CFM) for Optimization of Array CGH on Suboptimal Samples

Judy Chernos¹, Bob Argiropoulos¹, and Michael Chan¹

¹Cytogenetics Laboratory, Alberta Children's Hospital

Array CGH relies on good quality, high molecular weight genomic DNA for optimal results. We found that chromosomal microarray data for tissues (POCs and stillbirths) as well as prenatal samples yielded noisy data with many 'false-positive' CNV calls. We hypothesized that the difference in DNA fragment sizes between these tissues and controls was too great and affected hybridization rates and performance on the array. By developing an in-house method (CFM) for heat fragmenting control DNA to match fragment sizes of test samples we were able to produce

good clean data, with a reduced number of false-positive calls without the requirement of high molecular weight DNA.

Fun Cytogenetics of Chronic Lymphocytic Leukemia; Complexity, Dicentrics, Jumping Translocations and More.

Nyla A. Heerema, Ph.D.

Professor, Director of Cytogenetics, Department of Pathology The Ohio State University

Chronic Lymphocytic Leukemia (CLL) has a varied clinical course; some patients have an indolent disease, and others progress within months of diagnosis. One prognostic marker in CLL is cytogenetics. However, traditionally, the prognostic impact of cytogenetics has been limited to fluorescence in situ hybridization (FISH) because it was difficult to obtain dividing CLL cells in culture. This changed with the advent of CpG Oligodeoxynucleotide (ODN) stimulation, which results in ~80% abnormal karyotypes. Using CpG ODN stimulation of CLL in our routine clinical practice, we have found fascinating abnormalities in CLL, some of which have been shown to have prognostic significance. CLL has a very high level of cytogenetic complexity, and clonal evolution is common. Translocations, both balanced and unbalanced, are frequent. Interestingly, dicentric chromosomes are frequent; and jumping translocations, rare in other leukemias, are not uncommon. All of these phenomena are associated with loss of *TP53*, but also occur in the presence of *TP53*, so are not completely dependent on loss of *TP53*. All also have been associated with an adverse prognosis. This presentation will detail these interesting cytogenetic phenomena.