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GENETICS

G01. Complete Sequencing of TTR Gene in Brazilian Amyloidosis Patients

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Introduction: Familial amyloid polyneuropathy (FAP) is a dominant autosomal disease characterized by extracellular amyloid deposition in tissues of the body. Transthyretin (*TTR*) related FAP is the most frequent type of the disease. More than 100 mutations in *TTR* gene have been described, including the pathogenic variant c.148G>A p.Val50Met (legacy Val30Met), which is the most frequent mutation responsible for TTR-related FAP. The variant has multiple independent founder effect in different countries, including Portugal. Due to Portuguese colonization and high prevalence of Val30Met mutation in Brazil, a single Val30Met assay is currently used as an initial screening for patients having amyloid disease. In our diagnostic routine, we verified 51% positivity of Val30Met over 3 years, which motivated us to validate a test for entire sequencing of *TTR* gene, to search for other variants segregating in Brazilian FAP-patients. **Methods:** Four primer pairs were designed to cover all coding regions, 3´UTR, 5´UTR, and at least 15bp of intronic boundary sequence of *TTR* gene. Samples were sequenced using ABI 3500XL, and data analyzed using SeqScape software for coding regions and Bioedit program for UTRs and intron sequences. For validation, 16 samples with previous result were used for accuracy test and 05 samples were run in triplicate in different days and operators for reproducibility. In addition, 12 samples with FAP and negative for Val30Met were tested in order to search for other variants potentially correlated to the amyloidosis phenotype. **Results:** We found 100% correlation with previous results and 100% reproducibility in triplicate assays. In addition, the analyses of Val30Met negative patients showed the presence of a likely pathogenic variant c.200G>A, p.Gly67Glu (legacy p.Gly47Glu) segregating in 03 affected members of a family. We also found a variant in 3´UTR (c.*261C>T) in 2/19 cases, and an intronic variant (c.337-18G>C) in 01/19 cases. **Conclusions:** The complete sequencing of *TTR* gene showed reliable results and was implemented in our diagnostic routine to test patients negative for Val30Met mutation. The test will also be useful to describe a mutation profile of *TTR* gene in Brazil, as we found a mutation never described in Brazilian patients segregating in an affected family.

G02. Cancer Risk Assessment in Over 50 Thousand Individuals Carrying HFE Gene Variants for Type 1 Hereditary Hemochromatosis

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Introduction: Iron overload is a known carcinogenic factor and patients with hereditary hemochromatosis (HH) displaying increased iron overload indices, including serum iron, serum iron binding capacity, ferritin and transferrin saturation, are reported to have a 20-200-fold risk of intrahepatic cancer, namely hepatocellular carcinoma. In contrast, the reported risks for non-hepatobiliary cancers are conflicting and the data on the risk of cancer in heterozygous individuals (estimated allele frequency, 1/10 to 1/20) is limited. This study is aimed to better assess these risks in a large cohort. **Methods:** Using the Geisinger-Regeneron DiscovEHR cohort, we sequenced whole exomes of 51,289 study participants to further analyze the *HFE* gene variations (C2892Y, H63D or S65C), either in homozygous, compound heterozygous, carrier or WT status, for risk of cancer development. The cancer prevalence was assessed in both genders from multiple *HFE* genotypes as a percentage of a specific tumor within a genotype to be further compared to the WT individuals from the same gender. **Results:** Significant differences are noted in cancer prevalence in both genders

displaying *HFE* variants when compared to their WT correlates. Our data indicate that intrahepatic cancer (C282Y/C282Y: 5.85%; H63D/H63D: 1.12%; H63D/WT: 0.45%) and small & large intestine cancers (C282Y/WTY: 2.5%) are seen only in males, whereas, bone & cartilage (H63D/S65C: 1.72%), brain tumors (H63D/H63D: 1.72%) and esophageal tumors (H63D/S65C: 0.86%) are seen only in females. Malignant melanoma, other skin cancers, pancreas and lymphoid neoplasms can be noted in all carriers from both genders with differing percentages (3.45% - 1.29%). Gender specific tumors affecting only one gender are also noted; in males, penile (C282Y/H63D: 0.28%; all carriers: 0.2%-0.05%) and prostate cancers (all carriers: 12.3%-8.05%) show significant differences compared to WT. Similarly, in those cancers affecting only females, the prevalence is highest for uterine cancers (H63D/S65C: 4.3%), while tumors of the breast, ovary, cervix, vulva, and vagina are less frequently encountered in the HFE carriers (6.7%-0.23%). No significant increase in any cancer risk is identified in the C282Y/S65C group. **Conclusions**: To our knowledge this is the first study analyzing the *HFE* gene variants for cancer risk in over 50 thousand individuals following whole exome sequencing. While, C282Y and H63D homozygotes as well as compound heterozygotes (C282Y/H63D and H63D/S65C) revealed intra-hepatic cancers, skin tumors other than malignant melanoma, and/or brain tumors, the remainder of the cancers, especially those showing gender specificity, appeared to affect the carriers of the *HFE* variants with varying prevalence.

G03. Exome Sequencing of Breast Cancer Familial Pedigree and Functional Cellular Characterization Identifies Novel DNA Double-strand Break Repair Deficient Mutation

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Introduction: Mutations in the BRCA1 and BRCA2 genes have been the best characterized causal mechanism in hereditary breast cancer. However, disruptions to these 2 genes are only attributable for a quarter of the malignancies. **Methods**: We have sequenced the exomes of an Iranian non-BRCA breast cancer pedigree and have identified a shared truncation mutation in the RAD52 protein. **Results**: We have performed computational modelling and cellular assays to functionally characterize this truncation mutation and demonstrate it results in impaired double strand break repair and decreased DNA replication fork stability *in vitro*. **Conclusions**: This observation gives credence to the growing body of work regarding the complex involvement of RAD52 in breast cancer etiology and progression, and suggests that genetic background can modulate the effect of common or perceived "benign" mutations among different populations.

G04. Clinical Exome Re-analysis: Keeping up with Gene Discovery

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Introduction: Next generation sequencing has changed the landscape of clinical genomic analysis. With the explosion of genomic information and novel gene discoveries, clinical laboratories are faced with critical challenges in data interpretation. Since 2013, over 500 disease genes have been discovered via whole exome/genome sequencing. Over 250 phenotypes with a known genetic basis are added to Online Mendelian Inheritance in Man (OMIM) each year. Over 9,200 variants are added to the Human Genome Mutation Database per year. While this rapidly changing landscape improves the rate of molecular diagnoses, it also creates challenges. Initial clinical exome testing is nondiagnostic for 75% of cases. Re-analyses of these cases using the latest genetic information would identify additional molecular diagnoses but strategies to accomplish this in an efficient, scalable, sensitive, iterative, and cost effective manner has yet to be elucidated. **Methods:** The goal of this pilot project was to develop an infrastructure that enables iterative and sensitive identification of pathogenic variants in genes not known to cause disease at the time of initial analysis. To do so, we developed a patient data archive containing detailed patient phenotype data, including 2,000 clinician-selected Human Phenotype Ontology terms, chart reviews, patient genotype data, and all interpretive annotations for 130,000 variants generated during initial analysis. We next developed an algorithm, termed the Correlatron, which leverages our patient data archive and automates identification of potentially pathogenic variants in previously tested exomes. The Correlatron detects the phenotype-associated variants in each patient that are located in genes for which new information has been added to OMIM since the time of initial analysis. Also, the Correlatron facilitates regular iterative re-analysis of previously analyzed cases. **Results:** Two hundred-fifty clinical exomes initially analyzed between June 2014 and June 2016 were re-analyzed with Correlatron. Genes for all the 47 known positive cases were correctly identified. New molecular diagnoses were identified in 2.5% of cases. One example includes a phenotype match of developmental delay, microcephaly and cerebellar atrophy with a variant in *EMC1* supported by a recently published paper. **Conclusions:** For efficient re-analysis of genomic data, innovative strategies that leverage a well-developed infrastructure with complete phenotype and variant information are essential. Using clinical cases as examples, this study addresses the challenges and proposes strategies that clinical laboratories can utilize for re-analysis of genomic data, highlighting approaches for

systematic and sensitive re-evaluation of exome data that are both time and cost efficient.

G05. Establishment of a Rapid Genotyping System to Detect Obesity-associated Loci for Clinical Usage

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Introduction: Next-generation sequencing (NGS) is a high-throughput method for gene screening, and Sanger sequencing is the most common tool for genetic diagnosis. However, using Sanger sequencing or NGS were time-consuming, costly, and labor-intensive method. Thus, a rapid genotyping system to detect multiple genes or loci is necessary. Obesity is a global public problem because of obesity caused chronic diseases, such as type II diabetes mellitus. Environment factors and genetics were the major determinants of obesity. Many genetic loci associated with obesity markers such as Body Mass Index (BMI) and extreme or early-onset obesity were identified by genome-wide association studies (GWAS) of both European and Asian descent populations. To routine screening these obesity susceptibility loci, MALDI-TOF is a platform for rapid detection of multiple mutations and has been applied in clinical usages, such as *EGFR* mutation detection in lung cancer. In this study, we demonstrated that a mass dependent method was a sensitive, reliable, fast and cost-effective technique for clinical service. **Methods**: Sixty-six normal (BMI ≤24) and 80 adults with mild obesity (BMI ≥27) were collected from Lin-kou Chang Gung Memorial Hospital in Taiwan from 2014 ~2015. We performed an obesity gene panel including 137 SNPs located in 78 obesity-susceptibility loci by NGS to detect specific obesity-associated SNPs of Taiwan. Besides, a custom-designed MALDI-TOF assay using MassArray (Agena Bioscience) was developed to evaluate the finding from NGS. Fourteen mild obesity (BMI ≥27) cases were used to evaluate the performance of MALDI-TOF assay. **Results**: Fourteen obesity specific SNPs showed significant association with mild obesity from NGS data, which have been confirmed by Sanger sequencing. For clinical usage, 14 obesity-associated SNPs for Taiwanese people were designed in a custom-designed MALDI-TOF assay. A total of 88 wild types, 43 homozygous and 65 heterozygous variants were identified by MALDI-TOF assay in 14 SNP loci from 14 samples. The concordance of MALDI-TOF assay and NGS data is 100% for wild type, homozygous or heterozygous variants genotyping. The sensitivity, specificity and precision of obesity-MALDI-TOF assay were 100%. **Conclusions:** Lots of obesity-associated genes or SNPs were revealed. In this study, we showed that MALDI-TOF assay is a reliable, fast and cost-effective technique in routine molecular diagnostics. For the future, MALDI-TOF assay can apply in multiple genes or loci detection.

G06. ID4 Allelic Variant is Associated with Endometriosis and May Affect Mesothelial Epithelial to Mesenchymal Transition

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Introduction: Endometriosis affects approximately 10% of reproductive age women and is a leading cause of pelvic pain and infertility. This common disease is familial and genome wide association studies have suggested a potential risk locus called inhibitor of DNA binding (ID)-4 [rs6907340]. The ID4 minor allele variant (mav) is especially interesting to our group, because this tumor suppressor gene may be involved in the BRCA pathway and the epithelial-mesenchymal transition (EMT) process. **Methods:** Retrospective case: control study of all endometriosis cases diagnosed by tissue biopsy at OHSU from 2003-2010. Inclusion criteria required white non-Hispanic race (for allelic variant analysis), documented stage of disease severity (ASRM, stage 1 or 2 [mild], stage 3 or 4 [severe]), history of infertility (yes or no), and at least five years of documented clinical outcomes to evaluate for disease persistence and *de novo* presentation of breast cancer or adnexal cancer (ovary or fallopian tube). This yielded 180 cases for analysis. Negative controls were white non-Hispanic women with archived tissue biopsies for DNA and no history of endometriosis (n=177). DNA was extracted from FFPE tissue blocks and ID4 allelic discrimination was performed using Taqman. All samples were tested in duplicate and only reproducible genotypes were included for analysis. As a pilot study, we also immunostained a series of pelvic peritoneal biopsies for cytokeratin 7, E-cadherin, and N-cadherin, from endometriosis cases homozygous for the ID4 mav (n=7) compared with negative controls. **Results:** As expected, infertility was more common in cases of severe endometriosis (12/52, 23%) than cases of mild endometriosis (7/128, 5%), Fisher exact p=0.001. The ID4 minor allele frequency in white controls was 0.40 (expected 0.39). The frequency was increased in endometriosis (0.45) and more so in severe disease cases (0.47), yielding an odds ratio of 1.6 [0.99-2.75], p=0.05. The ID4 variant may also be a risk factor for breast/adnexal cancer (11% of CT/TT cases compared with 7% of CC homozygotes), odds ratio 1.82 [0.38-8.82]. In addition, our pilot IHC experiment suggested pelvic peritoneal mesothelial cells from cases may be more likely to lose E-cadherin staining and gain N-cadherin signal, consistent with EMT. **Conclusions:** Although the functional significance of the ID4 rs6907340 allelic variant is unknown, we observed significant differences in allele frequency related to the severity of endometriosis and potentially cancer risk.

G07. Genetic Screening for Chromosomal Abnormalities and Y Chromosome Microdeletions in 992 Korean Infertile Men

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Introduction: Infertility affects about 15% of couples of reproductive age, with about half of the cases involving male factors. Chromosomal abnormalities are confirmed as one of the frequent causes of male infertility. The microdeletion of the azoospermia factor (AZF) region in the Y chromosome was discovered as another frequent genetic cause associated with male infertility. The aim of this study was to evaluate the frequency and type of chromosomal abnormalities and Y chromosome microdeletions in Korean infertile men. **Methods:** A total of 992 infertile men were screened for chromosomal abnormalities and Y chromosome microdeletions. Cytogenetic analyses using G-banding and screening for Y chromosome microdeletion by multiplex PCR (LG Life Sceince, Korea) were performed in Korean patients with non-obstructive azoospermia and severe oligospermia (<5×106/ml sperm). **Results:** There was a high incidence (21.7%, 215/992) of chromosomal abnormalities and Y chromosome microdeletions in Korean infertile men. The overall prevalence of Y chromosome microdeletions was 9.2 % (91/992). Most microdeletions were in the AZFc region (51.3%) with a low incidence in AZFa (7.7 %) and AZFb (6.4 %). Larger microdeletions involving the AZFbc and AZFabc regions were detected in 26.9 % and 7.7 % of patients, respectively. Chromosomal abnormalities were detected in 147 patients (14.8%, 147/992). Klinefelter's syndrome was the most common (65.8%) followed by balanced translocation (12.7%) including 2 translocation of Y chromosome and autosome, Turner syndrome variant with Y structural rearrangement (11.4%) and Yq deletion (10.1%). Among the infertile men with Y chromosome microdeletions, the incidence of chromosomal abnormality was 24.2% (22/91). **Conclusions:** This study describes one of the largest studies of male infertility caused by Y chromosome microdeletions, especially in an Asian population. As such it is a significant contribution to the study of male infertility. These findings strongly suggest genetic screening for chromosomal abnormalities and Y chromosome microdeletions should be performed and genetic counseling should be provided before starting assisted reproductive technique.

G08. New Syndrome: First Patient with Heterozygous Deleterious PIG-Q Mutations Causing a GPI-anchor Biosynthesis Deficiency

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Introduction: Inherited congenital disorders of glycosylation (CDGs) include a subset of genes that are involved in glycosylphosphatidylinositol (GPI) anchor biosynthesis and attachment and, when damaged, lead to a variety of clinical features including intellectual disability and congenital anomalies. Collectively, the associated syndromes are referred to as PIG-X syndromes, with specificity derived from the precise gene involved in each individual syndrome. *PIGQ* (OMIM *605754) encodes phosphatidylinositol-glycan biosynthesis class Q (PIGQ) and is required for proper function of the N-acetylglucosomine (GlcNAc) transferase complex in a similar manner to the better studied PIGA, PIGC, and PIGH. PIGQ was shown to be critical for the GPI biosynthesis in *in vitro* studies as early as 2001, but only 2 patients have been proposed to have PIGQ disorders (both with consanguineous parents and homozygous mutations). **Methods:** A clinical genetics consultation and subsequent clinical whole exome sequencing were performed for diagnosis. Classification of identified variants were performed in accordance with the guidelines set forth by the American College of Medical Genetics and Genomics (ACMG). **Results:** Herein we describe the first patient to have compound heterozygous deleterious *PIGQ* mutations, including a maternally-derived frameshift mutation (c.968_969delTG) and a paternally-derived in-frame deletion (c.1199_1201delACT), causing a PIGQ-associated GPI biosynthesis deficiency. Our patient had dysmorphic features and multi-system involvement with ophthalmic anomalies, seizure disorder, cyclical vomiting, hepatic nodule, bilateral cystic renal dysplasia, prune-belly-like abdomen, and bilateral inguinal hernias. His skeletal anomalies were extensive and include unique radiolucent lesions in the bilateral proximal tibial metadiaphyses. **Conclusions:** The hallmark features previously reported in patients with PIG-X syndromes were observed in this patient. Given the historical convention for syndrome delineation in this subspeciality, we suggest that this patient provides evidence for a new syndrome, PIGQ syndrome. Additionally, this patient's multi-systemic involvement and skeletal anomalies expand the differential diagnosis of both cystic kidneys and radiolucent bone lesions in an infant.

G09. The Role of the Chemokine RANTES (Regulated On Activation, Normal T Cell Expressed and Secreted) as a New Diagnostic Tool in Monitoring Cardiac Adiposity

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Introduction: Epicardial adipose tissue (EAT), the visceral fat located around and infiltrating myocardium and great vessels, is considered an index of visceral adiposity and a promising indicator of high cardio-metabolic risk. EAT is a metabolically active organ able to produce many adipokines, cytokines and chemokines. Evidences showed that at increasing EAT the local production of these mediators is up-regulated and creates a condition of local chronic inflammation which could be the potential link between cardiac adiposity and cardiovascular risk. In the present study we aimed to quantify the gene expression of the chemokine RANTES and its receptor CCL5 in EAT samples and to explore whether local levels are related both to circulating levels and to cardiac adiposity. **Methods:** EAT biopsies and blood samples were collected from 23 obese and 9 non-obese patients undergoing open heart surgery for coronary artery bypass graft. A one-color microarray platform (Agilent Technologies, Germany) was used to evaluate gene expression in EAT. EAT thickness was measured by echocardiography using an M-mode color-Doppler VSF (Vingmed-System Five; General Electric, Horten, Norway) with a 2.5-3.5 MHztransducer probe. Plasma RANTES levels were measured by ELISA (R&D System, MN, USA). **Results:** Obese patients had higher EAT thickness (7.5 ± 0.9 vs. 5.4 ± 0.3 mm, $p<0.05$), higher levels of RANTES and its receptor in EAT ($p<0.05$ for both) and higher circulating RANTES levels (2469.9 ± 745.5 vs. 1272.1 ± 413.7 pg/mL, $p<0.05$) than non-obese. Furthermore, RANTES concentrations positively correlated with the EAT thickness ($p<0.01$). **Conclusions:** The parallel increase of local RANTES/CCL5 expression, circulating RANTES levels and cardiac adiposity may suggest that the elevated RANTES levels contribute to the local pro-inflammatory state and its quantification may be a useful indicator of cardiac adiposity and adiposity-related inflammation.

G10. Implementation of a New Automated Sample Quality Control Tool in a Whole Exome Sequencing Workflow

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Introduction: The German Cancer Research Center (DKFZ) is one of the largest biomedical research institutions in Germany. The High Throughput Sequencing Unit of the DKFZ Genomics and Proteomics Core Facility provides sequencing services for multiple applications. This project demonstrates the use of an automated electrophoresis system as a quality control (QC) tool in a whole exome sequencing workflow. Mandatory for the experimental success of whole exome sequencing is the quality of the incoming genomic DNA (gDNA) material and the DNA samples at various stages of the library preparation workflow. **Methods:** Exome libraries were prepared according to the Agilent Low Input SureSelect^{XT} Human All Exon v5 protocol from FFPE tumor tissue samples. The libraries were equimolar pooled. Each pool was sequenced on two lanes using the Illumina HiSeq 4000 System with 100 bp paired end sequencing. To ensure success quality control was verified with an Agilent 4200 TapeStation system of the received gDNA samples and during the library preparation. **Results:** Intermediate QC steps were taken throughout the protocol to monitor library preparation for sequencing, such as evaluation of DNA after fragmentation, analysis of adapter-ligated and amplified DNA, and lastly, qualification of the final library. The initial QC of incoming gDNA was determined based on the DNA integrity number (DIN). All samples had a low DNA integrity, what is usual for DNA extracted from FPPE material. Due to the low quality of the DNA material, a modified fragmentation protocol was used. Modification of the fragmentation enabled to obtain meaningful sequencing results. **Conclusions:** Quality control is an important part of NGS workflows, library preparation protocols recommend quantification and qualification of the DNA samples at various stages. The increasing sample throughput creates a need for automation especially in a core facility where many precious samples are proceeded with time pressure. The implementation of the automated electrophoresis system in the whole exome sequencing workflow enabled to increase the efficiency of the workflow and ensure good sequencing results.

G11. Genomic Dosage Anomalies in Circulating Tumor Cells from Early Stage Breast Cancer Serve a Surrogates for Anomalies in Primary Tumors

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Introduction: Currently, invasive procedures are necessary to obtain diagnostic tissue from suspected solid tissue tumor. Circulating tumor cells (CTCs), though not fully understood, serve as important mediators of metastatic disease and generally predict poor outcomes when present in high numbers. CTCs provide an opportunity to capture and characterize solid tumor profiles, potentially in the absence of invasive procedures. This study aimed to validate the use of a size exclusion filtering device to enrich for CTCs from peripheral blood and to determine the feasibility of downstream fluorescence *in situ* hybridization (FISH) on isolated CTCs through

comparison of their genomic profiles to that of the primary tumor. **Methods**: Patients with early stage breast cancer who were undergoing clinically-indicated resection or mastectomy and who had not been treated with neoadjuvant chemotherapy were consented for participation in the study. A core sample from the tumor and a peripheral blood sample were collected from each of five participants. DNA was extracted from the tumor following confirmation of lesional tissue by a pathologist, and somatic copy number variants (CNVs) were identified using the OncoScan FFPE Assay (Affymetrix, Santa Clara, CA, USA). Using CNVs identified in the tumor as loci of interest, fluorescence *in situ* hybridization (FISH) studies were performed on the peripheral blood sample following enrichment for CTCs using a size exclusion filtering device (ScreenCell, Westford, MA, USA). **Results**: No lesional tissue was available for one patient; no targetable CNVs were identified using the microarray platform for another. Of the remaining three patients, two patients exhibited atypical FISH patterns consistent with the CNV profiles generated by microarray (patient 1, loss of 9p21 and gain of 16p11.2; patient 2, abnormality of 8q24 including MYC), while no atypical cells were identified in the third patient using a FISH probe cocktail designed to target 3 CNVs observed in the primary tumor. **Conclusions**: The results of this study demonstrate success using a size exclusion filtering device to enrich for CTCs. These results also verify that the enriched cell fraction is compatible with downstream processes such as FISH. Although our cohort is small, these data suggest that tumor-specific somatic markers may be useful for interrogation of CTCs and that this process could play a critical role in identifying patients with a higher risk profile, even in early disease stages.

G12. Comparison of Buccal Specimens Collected with FLOQSwabs™ to Saliva and Blood for Genetic Investigations

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Introduction: Blood sample collection is very invasive and saliva device are difficult to use for children and not practical for donor screening. Buccal sample collection is noninvasive, easy and acceptable to children/adults and may increase donor participation to screening programs and can be used for collecting genetic material. Copan is producing hDNA free FLOQSwabs™, certified human amplifiable DNA, DNase and RNase free. The objectives of this study were to: Evaluate and compare the amount and quality of human DNA (hDNA) obtained from buccal swabs collected with hDNA free FLOQSwabs™ (FS) to saliva samples collected in Oragene (OR) and ORAcollect (OC) by DNA Genotek. Compare the amount of hDNA obtained from buccal swabs collected with FS to blood.
Methods: Buccal, saliva and blood samples from 20 volunteers were used. All volunteers collected 3 samples each in 3 different days, using FS and saliva samples in OR and OC as for collection methods provided by each manufacturer. After collection FS were stored in tubes with dry acting agents, while saliva was stored in the OR and OC saliva collection kits. Blood was collected from 2 donors to compare the recovery of hDNA. hDNA was extracted from each sample using QIAamp® DNA Mini Kit (Qiagen): the entire FS was eluted into the AL buffer; for OR and OC and blood ,200 ul of sample was added to the AL buffer and extracted as per kit procedure. Extracted hDNA was quantified form each sample by Real Time PCR (Quantifiler® Trio kit). Quality and purity of extracted DNA was measure by electrophoresis on agarose gel and by NanoDrop Spectrophotometer (Thermo Fisher).
Results: FS buccal samples gave an average of 50 ng/ul of hDNA (low 3.9, medium 22.6 and high 42.5 ng/ul of hDNA/swab) Average absorbance's at 260/280 of 2.01 (purity) and at 260/230 of 1.91 (impurity). Saliva in OR gave an average of 47 ng/ul (4700 ng of hDNA/200ul of liquid medium), average absorbance's at 260/280 of 2.04 (purity) and at 260/230 of 1.93 (impurity). Saliva in OC gave an average of 7 ng/ul (700 ng of hDNA/200ul of liquid sample and 3500ng of total hDNA /sponge), average absorbance's at 260/280 of 2.04 (purity) and at 260/230 of 1.73 (impurity). Blood gave an average 28 ng/ul (2800 ng of hDNA/200ul of blood), average absorbance's at 260/280 and at 2.18 (purity) and at 260/230 of 1.45 (impurity). No DNA degradation was detected on agarose gel with all samples analyzed. **Conclusions:** Data obtained in this study demonstrated that the Copan FS provided good quantity and quality of DNA compared to the OR and OC saliva devices. FS are non-invasive collection devices compared to blood samples, less expensive and easy to use compared to OR or OC saliva devices and can be used for buccal swabs collection for DNA genetic applications.

G13. Development of Robust PCR Assays and a Simple Analysis Solution for CYP2D6 Copy Number Assessment by High-Resolution Melting Analysis on Three Commercial Instruments

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Introduction: The cytochrome P450 2D6 (*CYP2D6*) gene encodes an enzyme that metabolizes one in four commonly prescribed drugs, including antidepressants, antiarrhythmic agents, and adrenergic antagonists. *CYP2D6* is located on the long arm of chromosome 22 with two nearby, highly homologous pseudogenes, *CYP2D7* and *CYP2D8*. *CYP2D6* is highly polymorphic and can also vary in copy number. Individuals with an ultra-rapid metabolizer phenotype can have three or more functional copies of the gene, whereas those with a poor metabolizer phenotype have zero functional copies of the gene. Rapid and accurate determination of *CYP2D6* copy number status is challenging but critical to properly

dose medications. We developed two multiplex PCR assays and a simple analysis solution to assess *CYP2D6* copy number variation (CNV). **Methods:** One of the developed assays determines copy number at intron 6. The other assay determines copy number at exon 9, the location of a well-documented *CYP2D6-CYP2D7* fusion event that results in a loss of function for *CYP2D6*. Each assay contained a primer pair specific for the target region within the *CYP2D6* gene and a primer pair for an ultra-conserved element (UCE). The UCE served as an internal control to normalize the PCR reactions. Genomic DNA samples with 0, 1, 2, 3, and 4 *CYP2D6* copies per genome were tested. The assays were performed with uniform PCR conditions, using both the deoxynucleotide triphosphates concentration and PCR cycle number as limiting factors, on three commercial thermocyclers. High resolution melting (HRM) analysis was performed using each instrument's corresponding software package. During data analysis, the melting temperature range was selected to include both the *CYP2D6* and UCE melting domains. The melt curves were normalized, and the software automatically clustered the results. A difference plot was used to determine the *CYP2D6* copy number for each sample, and the 2-copy *CYP2D6* curve was manually selected as the baseline. Accordingly, samples with 0 copies and 1 copy were clustered above the baseline, and samples with 3 or more copies were clustered below the baseline. **Results:** HRM analysis using difference plots accurately clustered the results in five distinct groups according to their *CYP2D6* copy number. For every sample, the *CYP2D6* copy number was concordant with the copy number as determined by digital PCR and other technologies. Assay performance was consistent across all three thermocyclers. **Conclusions:** We accurately detected *CYP2D6* copy number by developing two robust, multiplex PCR assays combined with HRM analysis. Our study demonstrates that using the difference plot available in commercial software packages is sufficient to assess *CYP2D6* CNV.

G14LB. Evaluation of a Home-brew NGS Assay for the Genetic Diagnosis of MODY Subtypes 1-3

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Introduction: Maturity Onset Diabetes of the Young (MODY) subtypes 1-3 account for the majority of clinically diagnosed MODY cases. A diverse spectrum of disease-causing mutations in the *HNF4A*, *GCK* and *HNF1A* genes have been reported in families affected with MODY over the years. A genetic diagnosis of MODY will result in a change of treatment, as there are established treatments that are catered for patients with MODY 1-3. On the basis of the prevalence and the implications of identifying persons with these mutations, the published clinical practice guidelines recommend that MODY genetic testing be performed only for these three subtypes unless there are clear non-diabetes related features suggesting other very rare causes. **Methods:** Five multiplex PCRs were designed to amplify the promoters, 5' untranslated regions and the entire coding regions of the HNF4A, GCK and *HNF1A* genes. Genomic DNA from 22 clinically-suspected MODY cases were subjected to these PCRs. Next-generation sequencing (NGS) was subsequently performed using the Illumina MiSeq platform on the libraries prepared from the amplicons. The Illumina VariantStudio v2.1 software was utilized for filtering and annotating the potential variants listed in the variant call format (VCF) files generated by the MiSeq Reporter v2.3. The pathogenic variants identified by NGS were validated with Sanger sequencing. **Results:** The 3 targeted genes are highly polymorphic. The supporting evidence of pathogenicity from *in silico* analysis was considered when interpreting the significance of novel missense variants. Of the 22 evaluated samples, 1 was identified with a novel missense variant (likely to be pathogenic) in the *HNF4A* gene, 2 were identified with pathogenic mutations in the *GCK* genes, and 1 was identified with a novel missense variant (likely to be pathogenic) in the *HNF1A* gene, respectively. These variants were confirmed to be true by Sanger sequencing. **Conclusions:** With this NGS-based method, screening for mutations in the relevant MODY genes is no longer impractical. This panel could be expanded by incorporating other candidate genes which are responsible for rarer MODY subtypes, or be supplemented by multiplex ligation-dependent probe amplification (MLPA) assay to enhance the diagnostic yield.

G15LB. Patterns of PML/RARA Gene Rearrangements by Interphase Fluorescence in Situ Hybridization in Acute Promyelocytic Leukemia

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Introduction: Acute promyelocytic leukemia (APL) is characterized by a reciprocal translocation t(15;17)(q22;q21) leading to the fusion of promyelocytic leukemia (*PML*) and retinoic acid receptor alpha (*RARA*) in 90% of the cases. Fluorescence in situ hybridization (FISH) has overcome the hurdles of unavailability of abnormal and/or lack of metaphase cells, and detection of cryptic, submicroscopic rearrangements. In the present study, we have explored the incidence of both typical and atypical FISH patterns of *PML/RARA* rearrangements by application of dual color, dual fusion DNA probe on interphase and metaphase cells and also compared FISH patterns to chromosome results in a series of 89 cases consecutive APL patients. **Methods:** During January 2010 to October 2016, a total of 89 APL patients who belonged to the age group 12-84 years were included in this study. Patients were diagnosed by standard morphology criteria and immunophenotype criteria. Conventional G-banded karyotyping was performed on bone marrow cells cultured for 24-hrs, and described according to the international system for human cytogenetics nomenclature. Interphase

FISH (iFISH) were performed using LSI dual color, dual fusion *PML/RARA* probe (Vysis Abbott Molecular Inc., Des Plaines, IL, USA) according to the manufacturer's instructions. **Results:** Our results showed that most patients with *PML/RARA* displayed typical iFISH patterns (n=62, 78.5%). Atypical iFISH patterns (n=17, 21.5%) were most frequently due to additional numerical changes – most often isochromosome 17q or duplication of derivative chromosome (der) of 17 (n=6, 7.6%), *RARA* translocation to *PML* on the der(15) followed by an insertion of residual *RARA* closed to *PML/RARA* on der(15) (n=6, 7.6%), or complex variant of 15;17 translocation with additional involvement of chromosome such as three way or four way translocations (n=2, 2.5%). One case showed *PML/RARA* rearrangement on iFISH but revealed normal karyotype, which indicated cryptic *PML/RARA* rearrangement. Secondary cytogenetic abnormalities were observed including trisomy 8. **Conclusions:** In summary, our results indicate that despite the high incidence of typical iFISH patterns of *PML/RARA* gene rearrangements, atypical patterns are also occasionally found in APL patients; the precise identification and characterization of the alteration present in individual cases is dependent on iFISH and chromosome analysis, and molecular study including reverse-transcriptase polymerase chain reaction could also be helpful.

G16LB. Analysis of Cell-free Plasma DNA for the Discrimination of Patients with Alcohol-induced Liver Cirrhosis from Liver Cancer Patients with a New Methylation Marker Panel

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Introduction: Chronic alcohol consumption is a strong risk factor for liver cancer and a variety of different factors contribute to alcohol-induced cancer development (i.e., carcinogenesis). Among these factors an aberrant pattern of DNA methylation seems to play an important role in the pathogenetic mechanisms leading to an alcohol-induced cancer development (Shukla and Lim Alcohol Res 2013). Recently the concept of "liquid biopsy" was introduced, comprising the analysis of extracellular or cell-free DNA (cfDNA) from different body fluids. Vaca-Paniagua et al (Epigenomics 2015) examined the methylation pattern of the *VIM* promoter sequence and found it to be different in cell-free DNA (cfDNA) from liver cancer (HCC) patients as compared to DNA from white blood cells originating from the same patients. In addition, Wen et al (Cell Res 2015) established a marker panel based on hypermethylated CpG islands for the detection of HCC patients in plasma DNA.The aim of our work is to generate and validate a new marker panel for the characterization of cfDNA able to discriminate cirrhosis patients from patients with HCC. **Methods:** In pilot studies we demonstrated that the methylation markers *mSHOX2* and *mSEPT9* are detectable in the supernatant of several HCC cell lines and in plasma of HCC patients with high specificity. In order to develop a better and HCC-specific biomarker panel several new real-time PCR multiplex assays were developed. We isolated and bisulfite converted plasma DNA from more than 150 cirrhosis and HCC patients using the commercially available Epi proColon Plasma Quick Kit. The newly developed biomarker panel was tested on these plasma samples and their performance compared to the results obtained with *mSHOX2* and *mSEPT9*. **Results:** In a first step we confirmed that the new markers are negative on plasma DNA from healthy subjects, and positive on DNA from tissue of HCC patients. Preliminary data let us assume that the new marker panel is specific and sensitive and able to discriminate between the 2 patient groups. **Conclusions:** We established a new multiplex assay that is able to discriminate HCC patients from patients with alcohol-induced liver cirrhosis. Before this method can be introduced into the clinical routine larger studies are needed to validate our data.

HEMATOPATHOLOGY

H01. Detection and Characterization of Genetic Aberration in Multiple Myeloma by Conventional and Molecular Cytogenetic Analyses

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Introduction: Conventional cytogenetic studies in multiple myeloma (MM) provide the advantage of whole genome analysis at one experiment. Combining fluorescence in situ hybridization (FISH) technology increases the number of cells analyzed, and allows detection of even submicroscopic abnormalities. This retrospective study aimed to investigate the genomic profiling in MM patients using conventional cytogenetic and interphase FISH analyses. **Methods**: This study enrolled 237 patients newly diagnosed with MM between January 2000 and October 2016. Chromosome analysis was performed on cultured bone marrow samples by standard G-banding technique. FISH panel of probes was used to identify genetic rearrangements, including CKS1B/CDKN2C, IGH/FGFR3, IGH/CCND3, IGH/CCND1, CEP12, D13S319, IGH, IGH/MAF, IGH/MAFB and TP53. At least 20 metaphase and 300 interphase cells were analyzed for each conventional cytogenetic and FISH studies, respectively. **Results**: Clonal chromosome abnormalities were detected in 107 of the 237 (45.1%) patients. 85 cases (79.4%) had both numerical and structural chromosome abnormalities. Overall hyperdiploidy with structural cytogenetic aberrations was the most common finding (43.0%), followed by hypodiploidy with cytogenetic aberrations (28.0%). The single most common abnormality was gains involving chromosome 1q (48.6%), followed by -13 or

del(13q) (39.3%) and 14q32 abnormalities (35.5%). By FISH analysis, representative abnormalities of IGH, 1q amplification, -13/del(13q), t(4;14) and t(11;14) were detected, respectively in decreasing order. **Conclusions**: While cytogenetic analysis can provide useful prognostic information, the low spontaneous proliferative activity of the tumor cells is considered to be a significant limiting factor. These limitation have been partly overcome by the use of molecular cytogenetic techniques such as FISH and comparative genomic hybridization (CGH). From the current study, combining conventional and molecular cytogenetic analyses, chromosome 1q gain, -13/del(13q) and IGH rearrangements are found to be the three most common aberrations in MM cases. Further prospective studies are needed to find high-risk genetic profiles and allow new risk-adjusted diagnoses and treatment for MM patients.

H02. Small Customizable Next-Generation Sequencing Based Target Capture Panels in a Clinical Environment Can Detect Variant Mutations at Frequencies as Low as 0.5%

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Introduction: Large scale hybridization panels are useful in clinical trials for novel therapies; they provide a plethora of information for targeted biomarkers. However, as drug targets are further characterized large panels can elicit a glut of information, compromising sensitivity in the selected biomarker subset. Therefore, once biomarker targets are identified, smaller hybridization panels can facilitate specific variant detection with greater sensitivity than larger gene panels and greater breadth than that available via PCR-based assays. Modifications of laboratory methods for small scale panels allow for the maintenance of high analytic quality with finely targeted panels. Our small panels (~10kb) focus on 1-4 genes, allowing for high-multiplexing of samples on sequencers, and reduced costs/processing times. **Methods:** Two separate next generation sequencing-target capture assays were developed with bioinformatics software under ISO13485 design control. One panel contained 3 genes, including fms related tyrosine kinase 3 (*FLT3*); the second covers only *CD274* (*PDL-1*). Libraries were made, hybridized with baits, and sequenced using the Illumina MiSeqDx. Validation was carried out by spiking in fixed amounts of mutant DNA into wild type DNA to establish the linearity and sensitivity of the assays. Sequencing libraries were generated by capturing with baits from either one or both panels. Sequencing data was analyzed using proprietary software developed by Invivoscribe. **Results:** DNA from 24 cell lines was assessed using both panels, confirming variants previously detected using other methods. A validation was run on the 3-gene panel using a series of contrived samples generated from cell lines containing between 0.5% and 25% variant allele frequencies for expected variants. Initial validation indicates that these small panel assays can detect mutations down to 0.5% variant allele frequencies. Assay linearity for *FLT3*/TKD detection from 0.25% to 12.5% or for *FLT3*/ITD detection from 0.5% to 25% is excellent (R^2= 0.996 and 0.998, respectively). Average sequencing coverage was high, ranging from 5,265x to 7,680x. **Conclusions:** Small hybridization panels are cost effective in detecting low-frequency variants from smaller subsets of genes while using far less DNA than individual PCR-based biomarker assays would require. These smaller assays focus on the most pertinent genes for a targeted therapy, and have the potential to greatly assist in understanding the molecular backgrounds of responders, super-responders, and non-responders, information which can help improve patient outcomes. Developing these assays with bioinformatics using the international ISO13485 design control standards makes them suitable for regulatory approval worldwide.

H03. Analysis and Characterization of Hematologic Cancers Using a Comprehensive NGS Panel Comprised of DNA and RNA Baits Targeting 704 Genes

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Introduction: As next-generation sequencing (NGS) methodologies improve, so does the ability to characterize hematopoietic and lymphoid neoplasm genomes. This promises to revolutionize oncology, allowing more accurate and precise classification of patients and potentially leading to novel targeted and combination therapies with improved outcomes. **Methods:** The MyHEME™ targeted sequencing panel is comprised of two independent bait sets that target a combined 704 genes known or predicted to contribute to hematologic cancers (DNA baits for 571 genes and RNA baits for 361 genes; 228 genes are found in common between the two bait sets). Libraries were constructed using 1µg of DNA or 0.1µg of RNA and sequenced on an Illumina platform. Sequenced reads are analyzed using proprietary MyInformatics™ software to identify single nucleotide variants (SNVs), indels and structural variants (SVs). Both the MyHEME panel and MyInformatics software were created under ISO13485 design control. To characterize the performance metrics of the MyHEME panel, we used the NIST human reference sample NA12878 along with combinations of hematologic cancer derived cell lines with known pathogenic variants at various allelic frequencies. **Results:** Analytical validation of the MyHEME panel established an average read depth of 1,175x (with a median read depth of 1,088x) for the DNA targets and an average transcripts per million (TPM) of 2,256 (with a median TPM of 743) for the RNA targets. For

the DNA targets, we establish sensitivity >95% (99.8% for SNVs at a 2.5% limit of detection (LOD); 100% for coding indels at a 5.0% LOD) and specificity >95% (95.5% for SNVs at a 2.5% LOD; 97.7% for coding indels at a 5.0% LOD). We also show the ability to cross-confirm results between the 228 genes common to both the DNA and RNA targets. Importantly, novel gene fusions, which are generally difficult to detect and validate, were cross-confirmed when observed in both the DNA and RNA targets. For example, we identified a novel t(9;22) translocation causing a *NUP214-XKR3* gene fusion using both the DNA and RNA targets. Additionally, while RNA data provides the fused exons of the transcripts, DNA data gives the precise genomic breakpoint coordinates. **Conclusions:** MyHEME is an extensive panel for sensitively and specifically identifying SNV, indel and SV mutations in 704 target genes. This panel can comprehensively characterize mutations in multiple diverse hematologic cancer samples, including AML, ALL, Non-Hodgkin Lymphoma and Multiple Myeloma. By utilizing a high depth of coverage, MyHEME can accurately detect clones present down to 5% of a patient's sample. In addition, by targeting both DNA and RNA, MyHEME contains a built in validation method to cross-confirm novel variants of interest.

H04. Assessment of Minimal Residual Disease by NGS in Acute Myeloid Leukemia Patients by Monitoring FLT3 and NPM1 Mutations

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Introduction: The molecular heterogeneity of acute myeloid leukemia (AML) has made a genetic-based classification essential for accurate diagnosis, prognostic stratification, monitoring minimal residual disease (MRD), and developing targeted therapies. Mutations in fms-related tyrosine kinase 3 (*FLT3*) and nucleophosmin (*NPM1*) genes are the most common mutations found in AML. The outcome of disease treatment of patients with *NPM1* mutation is influenced by the presence or absence of cooperating internal tandem duplications (ITD) in the *FLT3* gene. There is growing interest in prognostication through more extensive molecular profiling using the next-generation sequencing (NGS) technology. The development of sensitive and reliable assays to detect *FLT3* and *NPM1* mutations at very low frequencies for MRD represents a significant advancement in guiding treatment of AML patients. **Methods**: The NGS *FLT3*/ITD and *NPM1* MRD assays were designed to be compatible with the option to run both assays together. The *FLT3*/ITD assay targets exons 14 and 15 while the *NPM1* assay targets exon 12 of the respective gene. The DNA input for the assays was 700 ng (>100,000 cell equivalents). Up to 24 amplicons from either one or both assays were purified, pooled and sequenced before being analyzed using proprietary software developed by Invivoscribe. DNA isolated from bone marrow and peripheral blood from AML patients were tested by both assays. **Results**: Both *FLT3*/ITD MRD and *NPM1* MRD assays detected mutations with a sensitivity of 10^{-4} which equals one mutant cell in a background of ten thousand normal cells. The linearity of both assays are excellent in the read frequency range of 10^{-1} – 10^{-5}. Clinical diagnostic and follow-up samples were tested by *FLT3*/ITD MRD and *NPM1* MRD assays. The *FLT3*/ITD and *NPM1* mutations detected by MRD assays were the same as these detected by the capillary electrophoresis assay in diagnostic samples. In follow-up samples that were determined to be negative by the capillary electrophoresis assay, mutations with the same ITD length and sequence as the diagnostic samples were detected with the read frequency range of 10^{-3} – 10^{-6}. There was no detectable *FLT3*/ITD or *NPM1* mutation in some of the follow-up samples by MRD, which are concordant with clinical outcomes. **Conclusions**: The NGS *FLT3*/ITD MRD and *NPM1* MRD assays are highly specific and at least 2 orders of magnitude more sensitive than current commercially available assays. The results of clinical samples tested by the NGS MRD assays showed concordance with capillary electrophoresis assay results and clinical outcomes. The assays provide a reliable and cost effective tool to assess MRD in AML patients.

H05. Detection of Gene Amplification by Molecular Karyotyping in Acute Myeloid Leukemia with Marker Chromosomes

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Introduction: Gene amplification was frequently observed in marker chromosomes of AML and was associated with chromothripsis. Amplified oncogenes and chromothripsis may contribute to the tumorigenesis and progression of AML. Molecular karyotyping such as array CGH and FISH is useful to investigate the origin of marker chromosomes and complex chromosomal rearrangements. **Methods:** Marker chromosome is a structurally abnormal chromosome that cannot be unambiguously identified by conventional cytogenetics, and usually amplified or multiple rearranged chromosome material in cancer. Marker chromosome is rare in acute myeloid leukemia (AML), and is mostly accompanied by complex karyotype. We investigated the origin and characteristics of marker chromosomes in AML patients using array comparative genomic hybridization (CGH) and fluorescence in situ hybridization (FISH) analysis. **Results:** A total of 8 AML patients with complex chromosomal abnormalities and marker chromosomes were included in the study. The abnormal clones with marker chromosomes were more

than 30% of the total analyzed cells by conventional cytogenetic analysis of bone marrow. The array CGH analysis was performed by SurePrint G3 Human CGH 180K microarray (Agilent Technologies, USA) with DNA samples extracted from BM cells at diagnosis. Metaphase FISH was performed using BAC clones located within amplified regions. **Conclusions:** Complex karyotype of patients showed a median of 12.5 (6-27) chromosomal abnormalities with 1 to 4 marker chromosomes. Five patients had hypodiploid clones and the other 3 patients had hyperdiploid clones. From the array CGH analysis, the median number of copy number alterations was 11.5 (9-22). Copy number loss was frequently observed in 3p, 5q, 7q, 9q, 13q and 15q regions and copy number gain in 1p, 13q, 15q and 21q regions. Five patients had amplifications of 6p, 13q, 15q, and 21q originated from marker chromosomes, which were confirmed by FISH analysis. Amplified regions included oncogenes such as DAXX on 6p21.32, GPC5 on 13q31.3, and ERG on 21q22.2. The other 3 patients had marker chromosomes which were derived from a small part of a chromosome or rearrangement of several chromosomes. Three patients with gene amplifications had the multiple rearrangements of chromosomal segments, chromothripsis at 5q, 6p, 9p, 14q and 21q.

H06. Routine Somatic Hypermutation Assessment in CLL/SLL by Next Generation Sequencing Captures Multidimensional Clinically Relevant Data and Demonstrates Higher Sensitivity than Traditional Methods

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Introduction: Somatic hypermutation (SHM) status of the variable region of IGH (IGHV) is an independent prognostic marker in chronic lymphocytic leukemia (CLL). Routine assessment has traditionally relied on Sanger sequencing of PCR products using genomic or cDNA as templates. This approach is time/ labor intensive and may show limited sensitivity for low abundance clones. Here we report our experience using Next Generation Sequencing (NGS) for routine assessment of IGHV SHM. **Methods:** DNA from samples submitted for routine IGHV assessment was tested by our clinically validated NGS assay. PCR products using commercially available primers (LymphoTrack IGH FR1 kit, Invivoscribe) were sequenced on an Illumina MiSeq and analyzed by an in-house developed software, MSK-LymphoClone. SHM status was evaluated based on conventional criteria (mutation rate >2% compared to germline IGHV sequence). Full validation to establish accuracy, sensitivity, and reproducibility was previously performed comparing results to the reference method (PCR followed by Sanger sequencing, sensitivity 10%). **Results:** Validation encompassed 20 specimens (10 mutated, 10 unmutated). NGS testing showed 100% concordance with the reference method with respect to V-J usage of dominant productive clones and SHM status. Analytical sensitivity was 2.5% with excellent inter- and intra-assay reproducibility. In 5 samples (21% of patients), NGS detected a second unproductive clone in addition to the productive clone reported by the reference lab. Clinical assay performance was evaluated on 88 consecutive CLL patient specimens diagnosed over a 5-month period (4/2016-9/2016). A single dominant clone was detected in 66 patients (75%), 2 dominant clonal sequences detected in 21 (24%) and 3 independent clones in 1 patient. Among 22 patients with >1 dominant clonal sequences, 16 (73%) were predicted to have 1 productive and 1 non-productive IGHV, likely reflecting allelic exclusion. Two distinct productive clones were predicted in 5/22 (23%) patients, in 3 of these flow cytometry showed 2 distinct abnormal B cell populations. In 20/22 (91%) patients the SHM status of both clones was concordant (9 both mutated, 11 both unmutated), and in 2 patients SHM status was mixed. **Conclusions:** NGS-based assessment of IGHV demonstrates higher sensitivity than traditional methods and captures multidimensional clinically relevant data. The assay design significantly streamlines lab workflow allowing for batch sample analysis, simultaneous identification of unique clonal IGHV sequences (both productive and unproductive), and concurrent determination of SHM status. Routine NGS assessment in CLL reveals that biallelic rearrangement is more common than previously reported (25% vs 12% reported in the literature).

H07. ABC Subfamily C Member 10 (ABCC10) is a Promising Novel Target in Hodgkin's Lymphoma

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Introduction: Owing to the progress in its treatment, Hodgkin's lymphoma (HL) has become a potentially curable disease. However, there is a subset of HL patients has disease that is either refractory to treatment or relapses early; outcome for these groups is particularly poor. Moreover, patients receiving combined treatment are at higher risk for second malignancies. ABCC10, also known as multidrug-resistant protein 7 (MRP7), is the tenth member of the C subfamily of the ATP-binding cassette (ABC) superfamily. ABCC10 mediates multidrug resistance (MDR) in cancer cells by preventing the intracellular accumulation of certain antitumor drugs. The aim of this study was to determine the expression level of ABCC10 in HL cells and its potential prognostic/predictive significance. We also aimed to investigate ABCC10 reverasal effect on HL cells chemosensetization. **Methods:** Western Blott was used to delineate the protein level of ABCC10 in HL cell lines, Quantitative real-time PCR was conducted to determine the mRNA expression level of the target gene in both tumor cells and normal lymphocytes. We determined the effect of ABCC10 reverasal by its inhibitor "Tariquidar"

and its chemosensitization effect on HL cells by applying in-vitro proliferation assay. Immunohistochemistry was performed on HL paraffin sections to evaluate the expression of ABCC10 on primary HL tumor. **Results:** Results of our study showed that ABCC10 is over-expressed in most HL derived cell lines and primary HL tumor cells as compared to normal B cells. Our functional studies showed that inhibition of ABCC10 by one of its inhibitor (Tariquidar) had a significant dose-dependent increase in the sensitivity of HL cells to *doxorubicin*. In our study we also found that overexpression of ABCC10 was considered to be a negative prognostic factor for HL patients. We showed that there is a significant positive correlation between ABCC10 expression level in tumor cells and tumor stage, that in turn acts as a covariant, as it predicted initial response to treatment. **Conclusions:** These results indicate for the first time that ABCC10 plays a role in increasing toxicity of chemotherapy on HL cells, its overexpression affect clinical outcome, and it is a potential target in HL.

H08. FISH-negative, RT-PCR Positive PML-RARA a Rare, Infrequent Phenomena in Acute Promyelocytic Leukemia: A Series of 5 Cases

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Introduction: Rapid diagnosis and immediate treatment are critical and a requirement for favorable prognosis in Acute Promyelocytic Leukemia (APML). The diagnostic algorithm for APML arising from reciprocal t(15;17) relies on a multifaceted approach using morphology, immunophenotyping, cytogenetic and molecular analysis. Studies have shown that in APML t(15;17) can be detected by cytogenetics in virtually all cases of morphological APML. However, rare cases of FISH-negative, cytogenetically cryptic APML have been described in literature and failure in the diagnosis of these APML cases can lead to catastrophic outcome. Here we present a series of five FISH-negative, cytogenetically cryptic APML having classical morphologic and immunophenotypic feature diagnosed by RT-PCR at our center. **Methods:** This was a retrospective study of the APML cases from May 2012 to October 2016. All newly diagnosed cases of APML, referred to the cytogenetics and molecular laboratory were included and those negative for t(15;17) by FISH but positive for PML-RARA rearrangement by RT-PCR were analyzed. **Results:** Total 810 newly diagnosed cases of acute leukemia were assessed in the study period. APML constituted 6.7% (n=55/810) of the total diagnosed acute leukemia cases. Of the 55 APML cases diagnosed, 5 cases having characteristic morphologic and flowcytometry findings (CD34/HLA-DR negative; CD117, CD13, CD33 positive) of APML were found to be negative for FISH analysis of t(15;17). These cases were tested by RT-PCR for PML-RARA rearrangement and showed BCR1 transcripts. The mean age was 24 years with a range of 4-55 years and Male to Female ratio of 3:2. Survival data is available in 4/5 cases with a median follow-up of 22.2 months and 2/4 cases having low to intermediate risk disease remain in complete remission after 22-27 months of diagnosis at their last follow-up. Two cases had relapsed – one having high risk disease relapsed after 25 months of remission (died after relapse due to intracranial bleed) and the other having low to intermediate risk disease relapsed after 15 months of been in remission. **Conclusions:** Although cases of cryptic t(15;17) having normal karyotype have been infrequently reported in APML, fluorescence in situ hybridization (FISH)-negative cryptic PML-RARA rearrangement APML cases are even more rare, with only 35 such cases described in the literature till date to be best of our knowledge (largest series is of 10 cases with rest been case reports of 1 to 3 cases). We report here a series of 5 cases (n=5/55 APML-11%) which had characteristic morphologic and flowcytometry findings of APML but were FISH t(15:17) negative(n=5/5) and RT-PCR t(15:17) positive.

H09. Detecting Suspected B-cell Clonality Using an NGS LymphoTrack IGH Assay

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Introduction: PCR-based methods targeting immunoglobulin heavy chain (*IGH*) framework 1, 2, 3 (FR1, FR2, FR3), and joining region (JH) are the current gold standard for clonality testing in suspected B-cell proliferations. Recently, next-generation sequencing (NGS) based approaches for immune receptor genes have been suggested to improve sensitivity and identify the specific V-(D-)J DNA sequence required to track clones in follow-up testing. We developed comprehensive LymphoTrack *IGH* (FR1/FR2/FR3) Assays for both the Illumina® MiSeq® and ThermoFisher Scientific® Ion PGM™ platforms, which detect the vast majority of rearrangements in a single NGS run. Here, we report a pilot study of results of detecting suspected B-cell clonality using LymphoTrack *IGH* Assay. **Methods**: LymphoTrack® *IGH* Assay has been developed for both the MiSeq and PGM platforms. The proprietary V and J consensus primers were designed and adapted to enable the PCR products to be sequenced directly on either the MiSeq or PGM platform. Each MiSeq *IGH* FR master mix uses 24 indices, allowing analysis of 22 samples with 2 controls. Each PGM *IGH* FR master mix uses 12 indices, allowing analysis of 10 samples with 2 controls. One-step PCR was followed by amplicon purification. Purified equimolar amounts of amplicons were pooled to form a library, which was loaded onto the MiSeq or PGM. The

sequencing data was analyzed using Invivoscribe® LymphoTrack® bioinformatics software, which sorts the sequences by both index and FR, generate frequency distributions, and determine the somatic hypermutation (SHM) rate of FR1 amplicons. **Results**: The analytical performance of the LymphoTrack® *IGH* Assay on both platforms was evaluated using contrived samples with known V-J rearrangement. Both NGS assays demonstrated excellent linearity (R^2>0.99), sensitivity of 2.5% for clonality, and reproducibility (<20% CV). The clinical performance of the *IGH* Assays was evaluated on over 40 clinical samples that have been tested by control methods. Results demonstrated that clonality detection missed by one FR was detected by another FR, thus increasing overall detection rate. Both NGS assays demonstrated excellent concordance in detecting clonality in comparison to control methods. In addition, data generated with either the MiSeq or the PGM were concordant. **Conclusions**: A comprehensive *IGH* Assay has been developed for both MiSeq and PGM platforms that identifies clonal *IGH* V-J rearrangements and DNA sequences. Assay performance demonstrated that combining FR1, FR2 and FR3 helps to decrease the false-negative rate due to failure in amplification of any individual targeted FR region. The assay has demonstrated excellent concordance in detecting clonality in comparison to control methods.

H10. MYD88, A20 and CARD11 in HIV Associated B-cell Lymphomas

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Introduction: There is a paradigm shift in therapy in lymphomas from cytotoxic to targeted therapy with monoclonal antibodies like Rituximab and inhibitors of B cell signalling pathway. This study aims to analyze diffuse large B cell lymphoma (DLBL) and plasmablastic lymphomas in HIV patients for MYD88, CARD11 mutations and A20 to understand the abnormality in B cell signaling pathway. **Methods:** Forty-five HIV associated Non Hodgkins lymphomas plasmablastic and diffuse large B cell lymphoma activated B cell like as per Hans classification were retrieved from archives. Fluorescent in situ hybridization (FISH) was performed to detect TNFAIP3(A20) deletion and gene sequencing was performed to detect the MYD88 and CARD 11 mutations. **Results**: The study included 19 DLBL- ABC type, 18 Plasmablastic lymphoma(PL), 4 Gray zone lymphoma between DLBL and PL(n= 4) and 4 Gray zone between PL and Burkitt lymphoma. Only one (5.2%) DLBL showed MYD88 L265P mutation with A20 deletion while all other tumors were negative. CARD 11 exon 8 mutations were seen in 3(16.6%) PL. A20 was deleted in 1/11 PL and 1/ 5 DLBL where it was tested. **Conclusions:** MYD88 mutations are seen in HIV associated DLBL but they lacking in plasmablastic lymphoma indicating that chronic active BCR signaling pathway is more active. The CARD11 mutations described in this study in PL are interesting and will help us understand methods of targeted therapy in this aggressive lymphoma.

H11. Oligo-based Probes for the Rapid Detection of Lymphoma-associated Chromosomal Abnormalities in FFPE Samples

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Introduction: Fluorescence in situ hybridization (FISH) is a technique that allows for the visualization of common oncology related genomic aberrations, including gene deletions, amplifications, and translocations. This technique complements traditional cytogenetic analysis and immuno-histochemistry, and is a powerful method that is routinely used to assess diseases that result from chromosomal abnormalities, such as hematological malignancies. Lymphoma is a type of hematological malignancy and is characterized by several hallmark chromosomal translocations including those involving the *BCL2*, *BCL6*, *CCND1*, *IGH*, *MALT1*, and *MYC* genes. The presence of these chromosomal translocations function as biomarkers aiding in the stratification of lymphoma subtypes. To facilitate this characterization, both break-apart and dual-fusion probes were developed using Agilent's oligo technology to detect these gene rearrangements. We further developed a rapid 4-hour workflow for assessing these gene rearrangements in formalin fixed paraffin embedded (FFPE) tissue sections. **Methods:** The probes were designed using a novel algorithm to ensure maximum target coverage and avoidance of repetitive sequences and synthesized using Agilent's Oligonucleotide Library Synthesis methods. The probes were formulated in the rapid IQFISH hybridization buffer, providing a hybridization turnaround time of 90 minutes. Hybridizations were performed using both normal samples and samples harboring chromosomal rearrangements. Following probe hybridization, the probe signal intensity and signal patterns were assessed. Statistical analysis was used to assess the sensitive and specificity of the probes. **Results:** The probes had a sensitivity and specificity of >=98% and >=95% when assayed using normal metaphase spreads. When tested on FFPE samples, in all cases the probes were able to successfully classify normal and abnormal samples based on their differing signal patterns. For all abnormal cases, the detection of abnormal signal pattern demonstrated direct concordance (<20% diff) between Agilent's oligo-based probe and probes made using traditional, BAC-based methods. **Conclusions:** Combining Agilent's oligo probe technology with the rapid hybridization workflow enabled by the IQFISH buffer enables the robust and accurate identification of gene rearrangements commonly found in lymphoma.

H12. WITHDRAWN

H13. Frequency of MYD88 and CXCR4 Mutation Profiling in Lymphoplasmcytic Lymphoma/Waldenstrom's Macroglobulinaemia: Implications for the Molecular Laboratory

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Introduction: Recurrent mutations affecting Toll-like receptor signaling gene named Myeloid Differentiation Primary Response 88 *(MYD88)* and Chemokine receptor *(CXCR4)* have recently been described in Lymphomoplasmacytic lymphomas/Waldenstrom macroglobulinaemia (LPL/WM). *MYD88* L265P and *CXCR4* WHIM like frameshift and non-sense mutations in LPL/WM are of diagnostic /prognostic importance and use of inhibitors targeting these signaling pathways are being studied as novel targets for a rational approach to WM treatment. We share our experience of *MYD88* and *CXCR4* mutation analysis in LPL/WM cases referred to molecular laboratory at our center. **Methods:** This is partly retrospective and partly prospective study carried over a period of 5 years (2011 to 2015), and included 33 clinico-pathologically suspected cases of LPL/WM, and 1 case of CLL with raised IgM paraprotein. The mutation status and clinicopathological significance of these mutations was sought in 33 LPL/WM patients. *MYD88* hotspot mutation L265P and exon 5 were screened by Allele-Specific Polymerase chain reaction (AS-PCR) and Sanger Sequencing respectively where as *CXCR4* hot spot mutations (exon2) involving amino acids 295 to 352 were screened by Sanger Sequencing. **Results:** Of the total LPL/WM (n=33) cases screened, (84.8%, n=28) harbored *MYD88* L265P mutation by AS-PCR. This cohort had mean age 63.3yrs, M:F=2.3:1, median IPSS-WM 2.8 (n=23) and raised IgM (mean=4239.8mg/dl, range 561.5-11445mg/dl, n=25). All cases had anaemia (Hb :< 11.5gm/dl) and BM involvement by lymphoplasmacytic cells. Of the five L265P AS-PCR negative LPL cases, one case had IgA paraproteinemia (6973mg/dl), second had a differential diagnosis of WM/CLL(CD19, 5, 23, 22, 11c, Kappa restricted) with IgM paraproteinemia(4340.3mg/dl),the third LPL case had (IgM 4361.8 mg/dl. In 2 of the *MYD88* L265 mutation negative LPL cases no specific clinical details were available. Out of 28 *MYD 88* L265P positive cases on AS-PCR, 11 cases (39.3%) could be reconfirmed by Sanger sequencing and only two (2/28, 7.14%) had a *CXCR4* heterozygous non-sense mutation (p. Ser342*). Both these cases had neutropenia and aggressive clinical course. **Conclusions:** High frequency of *MYD88* mutations in suspected LPL/WM cases with intermediate to high IPSS-WM score co-related well with literature illustrating the role of this mutation in establishing the diagnosis of LPL/WM. AS-PCR as a highly sensitive method for MYD88 L265P mutation analysis could be of great help for the recognition of LPL/WM. Our data shows that the frequency of *CXCR4* mutated LPL cases is less than what is reported in literature. *MYD88* and *CXCR4* could form a basis for diagnosis and risk stratification in these low grade lymphomas.

H14LB. Prevalence of Alpha Thalassemia Mutations among Filipinos in a Tertiary Care Setting

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Introduction: Alpha thalassemia (AT) is a genetic defect characterized by a decrease or complete suppression of synthesis of the alpha globin polypeptide chains of hemoglobin. A recent local study showed an estimated AT carrier rate of 5 to 15% in the Philippines. The most common alpha thalassemia deletions are the Southeast Asia ($--^{SEA}$), Philippines ($--^{FIL}$), and Thailand ($--^{THAI}$) in Southeast Asia; the 3.7kb ($-\alpha^{3.7}$) and the 4.2kb ($-\alpha^{4.2}$) in the world. Currently, molecular characterization of AT is not routinely performed due to lack of financial resources. **Methods:** Peripheral blood counts, red-cell indexes and hemoglobin study results were retrieved from all patients with samples received for alpha globin genotyping. Genomic DNA was extracted from the peripheral blood, using the Qiamp DNA Blood Mini kit (Qiagen). The alpha chain genotype was analyzed using the alpha-globin strip assay (Vienna Laboratories). **Results:** A total of 220 blood samples were genotyped for alpha globin gene. The patient population was composed of 63 males and 157 females; 9 months old to 77 years old (mean: 39 years old). Of the 153 (70%) cases that tested positive for alpha globin chain mutation, 139 (91%) cases had single gene mutation, 14 (9%) cases had double gene mutation. The $-^{SEA}$ was the most common mutation (48%), followed by $-^{FIL}$ (37%), $-^{3.7}$ (6%), $-^{3.7}/-^{SEA}$ (5%). The remaining 4% of cases were composed of 2 cases of $-^{3.7}/-^{FIL}$, 2 cases of homozygous $--^{FIL}$, one case each of homozygous $--^{SEA}$, $-^{3.7}/-^{4.2}$, and one case of alpha 2 IVS 1-5 NT. Molecular characterization is limited to the variants tested in the alpha-globin strip assay. In our study, we were unable to characterize 30% of the underlying mutations causing thalassemia using the Vienna alpha globin chain strip assay. The Hemoglobin A2 levels and MCV were significantly lower in patients with double vs single alpha gene mutations (HbgA2, 1.51 vs 2.6, p<0.001; MCV 62.82 vs 67.39, p=0.03). The RDW was significantly higher in patients with double vs single alpha gene mutation (22.16 vs 16.34, p<0.001). There was no significant difference in the RBC and Mentzner index between two groups. **Conclusions:** We emphasize the importance of

doing molecular characterization of alpha thalassemia to improve management, control of disease and genetic counseling. We found that the three alpha thalassemia alleles make up more than 96% of the mutant alleles in our patients. Thus, a limited panel of DNA probes could significantly reduce economic burden and enable population-based screening more feasible. Further studies using full sequence analysis of the alpha globin gene are also needed to identify the less common mutations among Filipinos.

H15LB. Comprehensive Characterization of Targeted Sequencing Platforms to Elucidate AML-specific Mutational Landscapes

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Introduction: Routine molecular diagnostics in acute myeloid leukemia (AML) is presently based mainly on the concurrent screening of several genes for mutations of therapeutic and prognostic significance. Using a multiple single-gene assay approach is challenging, as such a large-scale analysis requires large amounts of DNA and is work and time-intensive. Next-generation sequencing-based platforms are now being tested for and introduced to highly parallelized detection of mutations in a clinical setting. However, a consensus has not yet been reached on which platform is the most suitable for a given situation. Here, we present a comprehensive comparison of four different NGS assays for AML diagnostics. **Methods:** We carried out targeted sequencing of 33 DNA samples from patients with AML and three replicate samples of Acrometrix Oncology Hotspot Control DNA each using the Ion Ampliseq Comprehensive Cancer panel (CCP) and the Ion Ampliseq AML Panel (AMLP) both from Thermo Fisher on the Ion Torrent Platform, the TruSight Myeloid Sequencing Panel (TSMP) from Illumina and the Myeloid Solution Panel (MSP) from Sophia Genetics on the Illumina platform. Data analysis comprising read mapping, realignment variant calling etc. was carried out using CLCBio Workbench, Ion Reporter Software (CCP and AMLP data), Somatic Variant Caller as implemented in Illumina BaseSpace (TSMP) and Genome Analysis Toolkit (GATK). Selected individual results were verified by conventional single-gene assays. **Results:** We were able to determine a wide range of performance characteristics of all 3 platforms. We show the extent of inter- and intra-platform variability for coverages, quality measures, variant calls, allele frequencies and other parameters. Especially, the Acrometrix Control samples allowed us to measure reproducibility and determine sensitivity and specificity of variant calling thus enabling us to fine-tune data analysis settings. We demonstrate that choosing the right data analysis software with settings adapted to the respective platform strongly enhanced the performance of all four assays (3 platforms). Furthermore, we were able to single out regions covered by the essays that performed in a suboptimal manner. **Conclusions:** Here we present the results of a comprehensive comparison of the properties of four major targeted sequencing platforms for AML. Although all 4 given assays can be used to elucidate the AML-specific mutational landscape of clinical samples, TSMP and CCP can also be used in a wider context and thus have different conceptual properties. The information given here should support individuals in making informed decisions on which platform to use given a distinct purpose.

INFECTIOUS DISEASES

ID01. Developing Amplification Control and Extraction Control for Vaginal Health Research Workflow

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Introduction: Imbalance of vaginal flora has been implicated in vaginal infections, which are associated in pre-term birth, infertility, and increased risk of sexually transmitted diseases (STD). Studies that enable profiling and monitoring vaginal flora help to understand susceptibility and risk factors for the vaginal health. Thermo Fisher Scientific has developed a large collection of TaqMan microbial assays to help researchers profile vaginal microbiota, which work well on both 384 well plates and high throughput OpenArray® platform. This assay collection targets 34 microorganisms (bacteria, fungi, protozoa and even virus) that are important in vaginal health, including normal ones present in vaginal flora and pathogenic ones. To enable researchers to better control major steps of their workflow, we designed and developed extraction controls for sample preparation and amplification control for quantitative PCR (qPCR). **Methods**: For amplification control, a DNA sequence is designed to cover the amplicons of all the assays in the collection and also several control assays. The synthesized DNA sequence is cloned into a plasmid vector, and the resulting superplasmid. After transformation and plasmid prep, the superplasmid quantified and then linearized. As a product, the superplasmid is normalized to 1X10^5 copies/ul for its final concentration. For extraction control, the synthesized DNA sequence (same as amplification control) was cloned into a yeast shuttle vector. Yeast competent cells are transformed with the resulting superplasmid. The grown yeast cells are inactivated, numerated and lyophilized. The yeast cells from extraction control go through sample prep protocol and tested on both 384 wells and OpenArray. **Results:** For amplification control, supercoiled and linearized superplasmids are compared. Our data show that a linearization of the superplasmid can lower Ct and increase replicate precision especially at lower concentration. The superplasmid control works well with

each of the assays on the panel. The performance of the superplasmid is also evaluated on Open Array by a serial dilution are also performed, at least 6 logs of linear dynamic range (with R^2 >0.99) and with limit of detection (LOD) down to ~50-100 copies or even lower. Good PCR efficiency and reproducibility were achieved. For extraction control, extracted DNA is also evaluated on Open Array. **Conclusions:** We have developed amplification control and extraction control for women's health research workflow. We demonstrate excellent performance and their utility for both controls. These 2 controls are useful for researchers to evaluate sample preparation and qPCR during their workflow and also can be used as valuable troubleshooting tools.

ID02. WITHDRAWN

ID03. Rapid Detection and Identification of Uveitis Pathogens by Qualitative Multiplex Real Time PCR followed by High Resolution Melting

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Introduction: Infectious uveitis is a serious sight-threatening intraocular infection that can be caused by a variety of agents. Distinct infectious etiologies may present with mixed clinical features that can lead to incorrect diagnostic and treatment. Complimentary laboratory tests are essential for the etiological diagnosis and proper clinical management. Here we explore the advantages of real time PCR technology and high resolution melting (HRM) analysis to develop a new, sensitive and reliable multiplex assay designed for rapid detection and identification of pathogens commonly associated with infectious uveitis, including cytomegalovirus (CMV), varicella-zoster virus (VZV), Epstein-Barr virus (EBV), herpes simplex 1 and 2 (HSV-1 and HSV-2), and *Toxoplasma gondii*. **Methods**: Genomic regions that yield PCR products ranging from 100 to 250 base pairs and with distinct melting profiles were identified in silico and selected for amplification. Analytical specificity, sensitivity and precision of HRM identification were determined using control genomic DNA. Clinical validation was performed by testing 149 intraocular fluids collected from eyes suffering with infectious uveitis (n=20) and controls (n=129) derived from eyes with bacterial endophthalmitis and non-infectious intraocular inflammation. **Results**: A distinct and non-overlapping melting profile was generated following HRM analysis with high precision (coefficient of T_m variation ≤0.02%). The T_m values for each target were: 79.2°C for *T. gondii*, 81.7°C for VZV, 84.0 °C for EBV, 85.7°C for CMV, 90.3°C for HSV-1 and 90.8°C for HSV-2. Cross-reaction with other potential intraocular pathogens not targeted in the assay was not observed. The assay was highly sensitive with a limit of detection of 40 genome copies/µL for most of the targets. Amplification efficiency ranged from 88% to 100%. Among 20 intraocular specimens collected from cases clinically suspected of having infectious uveitis, 12 were positive for one of the organisms tested. The mean Ct values varied from 18.7 to 29.7 with standard deviations no higher than 0.3. T_m values for the positive cases were compatible with HSV-2 (n=5), VZV (n=4), CMV (n=2) and *T. gondii* (n=1). Amplicon identities were confirmed by sequencing. All intraocular samples collected from control eyes were negative. The time for detection and identification of the most common uveitis pathogens in this homogenous assay was of approximately 1 hour and 40 minutes. **Conclusions:** This newly developed multiplex assay provides rapid, sensitive and reliable detection and identification of common uveitis pathogens and may represent a clinically useful diagnostic test to allow prompt and appropriate treatment of eyes affected by this sight-threatening disease.

ID04. Simultaneous Detection and Typing of Alpha, Beta and Gamma HPV Using Target Enrichment and Whole Genome Sequencing

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Introduction: Human Papillomaviruses (HPV) are a family of more than 200 DNA viruses classified into α, β, and γ genera that infect skin and mucosal surfaces. While most infections clear, persistent infection with some α HPV types is a risk factor for anogenital and oropharyngeal cancers. Current detection methods focus on α types with high risk. Multiple assays would be needed to detect all types in all genera. β and γ types have not been systematically studied. We use our recently developed universal HPV typing assay based on target enrichment and whole genome sequencing (eWGS) to detect and type α, β, and γ HPV in external genital samples previously examined by conventional typing restricted to 37 α HPV types (Roche Linear Array; LA). **Methods:** RNA bait targeting the full genome of 191 HPV types and human beta-globin (HBG, internal control) were custom designed and synthesized by Agilent Technologies. Samples included 50 residual anonymized DNA extracts (10ng-100ng) from external genital swabs. HPV-negative (100ng placental DNA and water) and HPV-positive controls (10ng SiHa DNA and 18 HPV plasmid DNA representing types 5, 6, 8, 11, 15, 16, 18, 20, 23, 24, 31, 33, 36, 45, 48, 52, 53, 58) were included. Library preparation and hybridization followed Agilent SureSelect XT2 protocol, and the library was sequenced on an Illumina HiSeq2500. CLC genomics workbench was used for sequence analysis. Type assignment was based on previously established stringent mapping conditions: cut-off of ≥1000 reads, coverage ≥20 and genome coverage ≥50%. **Results:** All samples with DNA generated an average of 10.5 million reads/sample with a mean Q score of 36.2; 93.4% of the bases had Q score ≥ 30. eWGS detected all 18 HPV plasmid positive controls (average depth ranged from 37 to 191, and the

fraction of reference covered ranged from 91% to 100%) and negative controls were negative. eWGS detected a total of 78 unique HPV types (45%, 27% and 28% of α, β, and γ HPVs respectively) in 32 cutaneous samples whereas LA detected 27 types from 27 samples, and all of them were α HPVs. Of the 51 unique HPV types detected by eWGS that were not identified by LA, 16%, 41% and 43% belonged to α, β, and γ HPVs respectively. The number of types detected by eWGS in test samples ranged from 1 to 22 (mean=4) with the most frequent types (≥3) being HPV84, 43, 90, 8, 120, 23, 115, 134, mKN1, mSD2 and 135. eWGS and LA results were concordant for the 37 LA types in 80% (40/50) of the samples. **Conclusions:** This eWGS method detects HPV types belonging to α, β, and γ in a single assay, without the use of degenerate PCR primers. Use of this method will clarify the importance of understudied HPV types.

ID05. COPAN eNATTM Stabilizes Fecal Bacterial gDNA in Warm Climates and is Compatible with Microbiome Profiling Using the GA-map™ Dysbiosis Test

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Introduction: Microbiome profiling has become increasingly common, its presence has a role for the gut microbiome in several human diseases, such as Crohn's, type-2 diabetes, autism, chronic allergies, and colorectal cancer. Proper storage of fecal samples for microbiota profiling is essential to avoid introducing post-collection bias in the microbial community composition. Rapid freezing, often not feasible, when sample collection is performed in warm climates, transportation is challenging, fecal bacterial composition changes rapidly at temperatures >25°C. A suitable transport medium that conserves DNA and stop bacteria replication may provide an alternative where refrigeration and cold chain transportation is not available. The GA-map™ Dysbiosis Test, the first CE-marked microbiota test, utilizes fecal material to identify and characterize dysbiosis in IBS and IBD patients. The test calculates a dysbiosis index (DI) score that indicates whether the microbiota pattern differs from what is found in a healthy gut. Sscore from -3 to +3 for selected bacterial is generated. To overcome the challenges of sample transportation in warmer climates, we examined the extent to which the stabilization buffers STAR from Roche eNAT™, MSwab™ from COPAN and another buffer maintained the composition of fecal microbial community structure at 40°C relative to freezing. **Methods:** Fecal samples from 5 donors were collected and transferred in triplicates into 4 storage systems; freezing or storage in either STAR, eNAT™, MSwab™ and buffer 4 systems at 40°C for 5 days. Upon storage, gDNA was extracted and analyzed according to the GA-map™ Dysbiosis Test protocol. The effects of storage in the different buffer systems at high temperatures on the dysbiosis index (DI) score and bacterial profile were analyzed. **Results:** The mean difference in DI scores between reference and test method, as well as the standard deviation and P-value in DI score between triplicates as in eNAT™ 0,09,0.10, and 0.067. MSwabTM -1.37, 0.13 AND 3.5*10-6, in STAR 0.21, 0.12 and 0.07, and in Buffer four 0.26, 15 and 0.09. eNAT™ was associated with very little alteration in DI score (0.09). Whereas in other buffers the samples had substantial divergence. The difference in bacteria profile of 15 selected genra and species between reference and test method. The results are given as the number of samples which a score change from individual probes. Storage in eNAT™ was associated with 16 changes, whereas samples stored in the other buffers showed a higher degree of divergence, 25.18 and 18 respectively. **Conclusions:** The Copan eNAT™ medium may provide an important alternative where refrigeration and cold chain transportation is not available.

ID06. MSwab™ Allows Viral Detection with Direct-rapid Nucleic Acids Amplification and Culture Assays

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Introduction: Molecular Rapid detection of viruses is important for patient care and can reduce spreading of infection. Extraction of nucleic acids can be costly and increases the results turnaround. MSwab™ is a molecular medium for the collection, and storage of clinical specimens for the detection of viruses with direct-rapid or traditional nucleic acid extraction and amplification assays and culture. It is compatible with in-house or commercial nucleic acids amplification assays and supports viral and bacterial viability for culture and antigens detection. Study objectives were to validate MSwab™ for- 1) Direct-rapid and traditional nucleic acids extraction for the detection of viruses by real-time PCR. 2) Virus isolation by shell-vial culture. **Methods:** Nasopharyngeal (N=80) and lesion swab (N=30) and saliva swabs (10) were used for this validation. Swabs from positive samples, first tested by real time PCR, including Flu A, Flu B, RSV, P1, P2, P3, Adeno, hMPV, HSV1, HSV2, and VZV were transferred in in a tube of MSwab medium. Saliva swabs were collected from patients and placed in a tube of MSwab™ medium. Each MSwab™ tube was vortexed and 200 ul of sample were used to inoculate a shell vial culture, another 200 ul were added to a microtube and placed in a dry heating block at 1000C for 5min, vortexed for 10s and centrifuged at 14,000rpm for 2min. A 200ul aliquot was extracted with the easyMagTM (Biomerieux) and eluted in 55ul. Five ul of each nucleic acid extracted with both methods were tested with the in-house singe or multiplex real time PCR and

compared to the UTM results. **Results:** MSwab direct-rapid and EasyMag nucleic acid extraction tested by real-time PCR and shell-vial culture confirmed all FA FB, RSV, P1, P2, P3, ADV, HMPV, HSV1, HSV2, VZV and CMV. No toxicity or contamination was observed in culture. **Conclusions:** The data obtained in this study demonstrated that MSwab™ can be used for direct-rapid nucleic acid extraction for the detection of viruses using single or multiplex real-time PCR and viral culture confirmation of new influenza strains or for antiviral resistance. MSwab™ direct-rapid extraction improves results turnaround time, save costly extraction reagents and supports culture confirmation.

ID07. Characterization of Clinical Specimens Using GenMark's ePlex® Blood Culture Identification (BCID) Panels

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Introduction: Bacteremia and sepsis constitute major health and financial burdens in the United States and internationally.(1) Rapid diagnosis of bloodstream infections remains a challenge for the early targeting of antibiotic therapy in sepsis patients.(2) GenMark is developing a broad blood culture identification (BCID) menu which includes three comprehensive panels for the identification of fungi (BCID-FP), gram-positive bacteria (BCID-GP) and gram-negative bacteria (BCID-GN) and associated genetic resistance determinants from positive blood culture after a Gram stain has been performed. Each panel is a single-use cartridge that contains all reagents required to enable sample-to-answer testing, including extraction, amplification, and detection via GenMark's proprietary eSensor® technology. The objective of this study was to characterize residual clinical samples from patients with single or multiple infections using the ePlex BCID Panels and compare the ePlex result to the reported standard of care result. The ePlex BCID panels are currently under development. **Methods:** Based on known Gram stain results, 242 samples were tested on the appropriate ePlex BCID panel and results were compared with the reported standard of care result. Bi-directional sequencing, culture, or qPCR were used for discrepant resolution. **Results:** Ninety-three retrospective gram-positive (GP) clinical samples were evaluated, including single and polymicrobial infections. Overall initial concordance was 89.2% with the reported result and 97.8% following discordant resolution. Ninety-five retrospective gram-negative (GN) clinical samples evaluated were 93.6% concordant with the reported result and 97.8% after discordant resolution. Fifty-four retrospective fungal clinical samples were 100% concordant with the reported result after discordant resolution. Forty-five polymicrobial infections were evaluated across two or more of the panels; after discordant resolution, 95.5% of the samples tested were concordant. **Conclusions:** The ePlex BCID panels were able to detect a broad range of sepsis causing fungal pathogens and GN and GP bacterial pathogens along with their associated resistant markers. ePlex BCID panels were also able to accurately detect polymicrobial infections, including samples with mixed GP/GN, GP/FP, and GN/FP pathogens. Rapid identification of bacterial and fungal pathogens responsible for sepsis can assist physicians in earlier diagnosis and targeted treatment, which is associated with improved outcomes and reduced healthcare costs.(3,4)

ID08. Clinical Evaluation of a Novel NGS-based HIV-1 Drug Resistance Monitoring Test

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Introduction: The worldwide scale-up of antiretroviral therapy has decreased HIV mortality and improved clinical outcomes. However, incomplete suppression is known to be a risk factor for developing Drug Resistance Mutations (DRMs). The objective of this study was to evaluate a novel Next Generation Sequencing (NGS)-based HIV-1 drug resistance monitoring test (*Sentosa* SQ HIV-1 Genotyping Assay). **Methods:** In this study we used an automated NGS-based integrated workflow, comprised of 1) a robotic liquid handling system for RNA extraction and NGS library preparation (*Sentosa* SX101); 2) Ion Torrent's instruments for deep sequencing; 3) kits for RNA extraction, HIV NGS library preparation and sequencing, and 4) data analysis and reporting software. Reporting includes 276 amino acid (AA) mutations in 103 AA positions across the Reverse Transcriptase (RT), Protease (PR) and Integrase HIV genes. **Results:** 3,330 prospective clinical samples from patents infected with HIV-1 were tested on Vela's *Sentosa* SQ NGS system. 91.9% (3061) of the samples were subtyped as CRF01_AE, 3.7% (124) as recombinant form BC, 2.5% (83) as subtype B, <0.4% (12) as other subtypes. Fifty samples (1.5%) were infected with 2 or more HIV-1 strains belonging to different subtypes. In total, 33,439 DRMs were detected in 3,330 samples (15,789, 17,226 and 424 in the RT, PR and Integrase genes respectively). These DRMs were distributed across the target genes as follows: 94 AA variants in 31 AA positions of the RT gene, 78 AA variants in 38 AA positions of the PR gene and 32 AA variants in 23 AA positions of the Integrase gene. The most prevalent DRMs in the RT gene were: K238R(53.1%), M184V(47.5%), K103N(35.9%), Y181C(26.8%), V179I(25.1%), G190A(18.4%), D67N(14.5%), K65R(14.4%), K103E(13.8%), H221Y(12.5%), K101E(12.0%),

N348I(11.0%), T69N(10.6%), V106I(10.0%). In the PR gene: M36I(94.2%), H69K(93.6%), L89M(85.5%), G16E(35.2%), L63P(30.8%), K20R(27.9%), L10I(22.3%), I93L(21.9%), L10V(16.5%), I62V(13.4%), V82I(11.5%), and in the Integrase gene: S230N(2.7%), V151I(2.3%), E157Q(1.6%), L74M(1.2%). **Conclusions:** To our knowledge this is the first large clinical data set employing automated Ion Torrent based NGS as the analytical method for detection of DRMs in HIV-1. NGS has demonstrated clinical utility by providing results in a comparatively short period of time with increased sensitivity compared to conventional sequencing approaches. The new NGS workflow thus appears as a promising new tool for detecting clinically relevant variants in HIV-1.

ID09. Comparison of Accupower TB&MDR Kit with Hain Genotype MTBDRPlus Kit for Detection of Mycobacterium Tuberculosis Drug Susceptibility in Pulmonary Samples

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Introduction: Tuberculosis (TB) is the leading cause of mortality in adults due to M. tuberculosis and accounts for 26% of all preventable adult deaths globally. Rapid molecular techniques involved in the assay of MTB drug resistance detect the sensitivity of the mutations in two genes, Rifampicin (RIF) and Isoniazid (INH). RIF resistance is due to mutation of the rpoB gene (81bp). INH resistance is caused by mutations in katG gene. Analysis of strains collected in different countries show different prevalence of the mutations. A lot of previously studies regarding the three frontline drugs in tuberculosis chemotherapy with the loci rpoB, katG, inhA were characterized by strip based Hain Genotype MTBDRPlus Hybridization method. Here we have compared the characterization and analysis of rpoB, katG and inhA loci by Hain MTBDRPlus Hybridization (Hain) and Real-Time PCR by Accupower TB&MDR kit (Accupower) provided by Bioneer Corporation, South Korea.
Methods: A total 133 pulmonary Sputum and Pleural fluid samples were referred to laboratory of which 120 samples (93 Sputum, 27 Pleural Fluid) were selected randomly for TB Drug susceptibility test. All samples were subjected to homogenization, decontamination by NALC and NaOH method. Smear positive were tested for drug resistance. Each sample was segregated and extracted separately using Hain and Accupower assays as per recommended protocols. **Results:** A total of 120 positive pulmonary TB patient samples with M. tuberculosis were obtained. 94 samples were positive for TB in Hain assay whereas 102 samples showed positivity for TB by Accupower. Eleven patients with positive smear test has not shown any amplification in both assays. Sensitivity for RIF was shown in 45 & 42 samples resp.in Hain and Accupower. A total of 43 samples were sensitive and 37 were resistant for both RIF and INH respectively by Hain. By Accupower assay, 33 samples and 40 samples were sensitive and resistance for both RIF and INH respectively. RIF resistance and INH sensitive by either assays was seen in 12 and 20 samples. Resistance to the RIF was detected in 49 (52.1%) and 60 (58.8%) samples by Hain and Accupower assays respectively. INH resistance was observed in 39 (41.4%) and 49 (48%) samples respectively. 38 samples (38/39, 97.4 %) were correlated for INH resistance by both the assays (inh A-6, Kat G 315-31, inh A+Kat G 315-1). The remaining ten were not correlated. The detection of TB by Accupower with 93.5% is more compared to the Hain 86.2%. The resistance pattern for both RIF and INH is more by Accupower whereas the sensitive pattern is nearly equal by both methods. **Conclusions:** Real Time PCR based Accupower TB&MDR has more diagnostic value in detection and convenience of use compared to Strip based method by Hain GenoType.

ID10. Clinical Performance Evaluation of Lyophilized VIASURE Real Time PCR Detection Kits for Pathogen Detection by Participation in External Quality Assessment (EQA) Programs

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Introduction: VIASURE provides real time monoplex and multiplex assays that allow the simultaneous detection of viruses, bacteria, parasites and fungi considered the major causal agents of respiratory, gastrointestinal and tropical infections. Unlike most commercial test available in liquid format, these kits contain all the necessary components for the qPCR assay in a stabilized format. QCMD and INSTAND are independent organizations offering External Quality Assessment (EQA) programs that enable laboratories to benchmark, monitor and improve the quality of their molecular diagnostic technologies and routines. During the last year, CerTest Biotect has participated in 29 EQA programs (16 from QCMD and 13 from INSTAND). In addition to these original purposes, this study allows us to assess clinical diagnostic accuracy of VIASURE kits against expected results and those obtained by additional commercial kits and verify the advantage of using lyophilized qPCR reaction mix. **Methods:** A total of 255 samples from QCMD and INSTAND panels have been evaluated by VIASURE assays. Briefly, DNA/RNA were isolated using VIASURE RNA-DNA Extraction kit (CerTest), amplified and detected with 39 VIASURE kits in accordance to the instructions of the supplier (Certest) on Bio-Rad CFX96™ Real-Time PCR Detection System. These test are intended for use as an aid in the diagnosis of the following respiratory pathogens (Influenza A, H1N1, and B, Metapneumovirus, Parainfluenza virus, RSV, *Legionella*, *Bordetella, P. jirovecii*); gastrointestinal specimens (Adenovirus, Rotavirus, Astrovirus, Norovirus, Sapovirus, *Campylobacter*, *C. difficile, H. pylori, Salmonella*, *Shigella*/EIEC and *Y. enterocolitica*, *Cryptosporidium*, *G. lamblia*, *E.*

histolytica and *dispar*) and tropical fever viruses (Chikungunya, Dengue, West Nile and Zika). **Results:** VIASURE has tested 68 respiratory, 76 gastroenteritis and 39 tropical fever samples from QCMD panels. 96.1% (176) have been successfully detected. For *Legionella* spp. assay we obtained 1 false positive, 2 were educational samples re-tested and correctly confirmed as Norovirus positive and 4 were Tox A negative samples. From INSTAND panels 72 specimes were evaluated (30 respiratory, 20 fecal and 22 tropical specimens). 98.6% (71) have been successfully detected. The incorrect sample was re-tested and correctly confirmed as *H. pylori* negative. In addition, Dengue Serotyping kit correctly identified serotype 1, 2 & 4. **Conclusions:** VIASURE kits consistently scores high levels of accuracy in assessments, across all syndromic groups. VIASURE lyophilized assays are high quality reliable and show higher sensitive, specific and consistent than classic liquid format test.

ID11. Comparison of Standard Reduced Transport Fluid (RTF) Buffer to the New eNAT Buffer Shows Vaginal Microbiome Stability for up to One Month in eNAT Buffer as Determined by the ISpro Microbiome Profiling

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Introduction: Current collection buffers for microbiome analyses need to be stored at 4C before sampling and frozen after taking the sample. This makes collection in rural areas and situations of delayed freezing unreliable. In the current study we compared standard reduced transport fluid (RTF) buffer to the new eNAT buffer which can be stored at room temperature for up to 1 month after sampling. The study was performed as part of the ReceptIVFity study in part funded by the Eurostars project VALBIOME. M**ethods:** Ten vaginal swabs collected in either standard RTF buffer or eNAT buffer (Copan) were analyzed for their vaginal microbiome composition by IS-pro profiling. Samples in RTF buffer were frozen after sampling and transferred to the laboratory for analyses. Samples in eNAT buffer were kept at room temperature and shipped at room temperature to the laboratory. Samples collected in eNAT buffer were compared for their profile 3 days, 1, 2 and 4 weeks after collection and storage at room temperature to the profile generated for the same patient sample in RFT buffer and stored at -20C. **Results:** The IS-pro profiles generated for the samples in RTF buffer were equal to the profiles of the identical patient collected in eNAT buffer. In addition, the profile for 2 days and 1, 2 and 4 weeks were highly similar, no additional bacterial peaks appeared and no peaks were lost in time during the storage of 1 month at room temperature. **Conclusions:** This pilot experiment shows clearly that swabs stored in eNAT buffer can be used reliably for analyzing the vaginal microbiome by IS-pro after up to one month of storage at room temperature. This enhances study opportunities in rural areas and situations with potential delayed freezing of the samples. Currently we are in progress analyzing 50 vaginal swabs collected in RTF versus eNAT buffer with a comparison after 4 weeks to further validate our pilot data. This study will be ready in the first quarter of 2017 and presented at the meeting.

ID12LB. Diagnostic Utility of Real-Time PCR for Mycobacterium tuberculosis Complex Infection in Routine Clinical Practice

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Introduction: Rapid detection of *Mycobacterium tuberculosis* (MTB) is important for early diagnosis of tuberculosis in a patient and for prevention of pathogen transmission to another person. Due to delayed growth of MTB in culture media and low sensitivity of acid-fast bacilli (AFB) smear, polymerase chain reaction (PCR)-based DNA amplification techniques have been increasingly used for MTB detection in clinical practice. Especially, real-time PCR has advantages of high sensitivity, short analysis time, and reduced chance for cross-contamination between samples. The aim of this study was to evaluate the clinical utility of real-time PCR for MTB detection in respiratory specimens. **Methods:** During June to September 2016, a total of 283 respiratory specimens from 283 patients who were suspected of tuberculosis were included in this study. Using digested and decontaminated specimen, DNA was extracted using QIAamp Blood Mini Kit (Qiagen, Hilden, Germany) with QIAcube System (Qiagen). The PowerCheck MTB/NTM Real-time PCR assay (Kogene Biotech, Seoul, Korea) includes primers targeting *IS6110* sequence of MTB and the internal transcribed spacer sequence common to all mycobacteria. PCR-amplification and detection was performed using the CFX-96 Real-Time PCR Detection System (Bio-Rad Laboratories Inc., Hercules, CA, USA). Results from the real-time PCR were compared with results from AFB smear and mycobacterial culture. Culture-negative specimens were confirmed using culture results from follow-up specimens and final clinical diagnosis. The statistical analysis was performed using SPSS Statistics version 24.0.0 (IBM Corp., Armonk, NY, USA). **Results:** Among 283 patients, 26 (9.2%) were diagnosed with pulmonary tuberculosis. When the results were compared with final diagnosis, the sensitivity, specificity, and positive and negative predictive values were 73.1%, 99.2%, 90.5%, and 97.3% for the real-time PCR, 57.7%, 99.2%, 88.2%, and 95.9% for AFB smear, and 92.3%, 100%, 100%, and 99.2% for mycobacterial culture. On average, the real-time PCR results were reported the next day of specimen receipt in the laboratory. **Conclusions:** The PowerCheck

real-time PCR assay showed higher sensitivity than AFB smear and much shorter turnaround time than mycobacterial culture. Our data demonstrated that the real-time PCR assay is useful in conjunction with conventional methods; however, careful and combined interpretation with other laboratory tests should be needed to diagnosis tuberculosis. In conclusion, the real-time PCR assay could be used as useful method for rapid diagnosis and control of tuberculosis.

ID13LB. Usefulness of Molecular Test to Diagnose EBV Infection in Immunocompetent Patients

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Introduction: Epstein-Barr virus (EBV) is one of the most common infectious viruses in humans. EBV is the causative agent of infectious mononucleosis but is also associated with various clinical conditions such as hepatitis, hematologic, and solid cancers. Serological tests for EBV were frequently used for diagnosis of EBV infectious status, clinicians use the tests with confidence. However molecular testing should be needed for accurate diagnosis for EBV infection. **Methods**: We reviewed laboratory records of routine blood specimens requested for EBV viral capsid antigen (VCA) IgM and IgG antibodies, and IgG antibody against the EBV nuclear antigen (EBNA), retrospectively. Total 279 specimens from immunocompetent patients were selected, and EBV DNA tests (artus EBV RG PCR Kit, Qiagen, Germany) and additional EBV serologic assays (ARCHITECT EBV assays, Abbott, Germany; DiaSorin Liaison EBV assay, DiaSorin, Italy; PLATELIA EBV assay, Bio-Rad, France) were performed for all specimens. **Results**: Seventy-four specimens were positive for EBV DNA. VCA IgM Positive from more than two EBV serological tests were resulted in 14 specimens, 1 of 14 was negative for EBV DNA. Specimens with EBV DNA positive and negative VCA IgM were 61. Four of 61 were positive for VCA IgM at least one EBV serological test. **Conclusions**: Acute primary or transient EBV infections were decided when VCA IgM antibody positive from more than two EBV serological tests regardless of VCA IgG and EBNA IgG results. One specimen with negative for EBV DNA and positive for VCA IgM was considered because of an impact of Herpesviridae latency on molecular testing. Sensitivity differences of methods could explain results of 61 specimens. Our results suggest that EBV molecular test should be performed for EBV infection diagnosis even in immunocompetent patients.

INFORMATICS

I01 Withdrawn

I02. A Bayesian Network Algorithm for the Identification of Cancer Somatic Mutations in the Lack of Normal Tissue from Targeted High-throughput Sequencing Data

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Introduction: Tumor molecular profiling is rapidly becoming the standard clinical test for selecting targeted therapies in refractory cancer patients. DNA extracted from patient samples is enriched for cancer genes and sequenced to identify actionable somatic mutations therein. A major challenge arises when tumor-derived data is analyzed in the absence of normal tissue data, as it is common in clinical scenarios. The distinction between somatic and germline variants become difficult, leaving clinicians to resort to crude heuristic filtering. We present here a variant calling software capable of accurately identifying somatic mutations from targeted next-generation sequencing data. **Methods:** A novel Bayesian network approach models the distribution of reads harboring germline and somatic mutations, estimates the contamination from normal tissue in the sample, scores somatic mutations, and imputes germline variants, without matching normal tissue data. To improve specificity, our caller can also utilize prior information from different databases including somatic mutations (e.g. COSMIC) and germline variation (e.g. ExAC) in a principled fashion. In the absence of normal tissue, our method scores each variant with respect to their likelihood of being somatic or germline and this score can be used for creating filtering cut-offs that deliver only likely somatic mutations, whereas imputed germline variants output can be also produced. **Results:** We assessed our method with semi-simulated data where artificial somatic mutations were spiked at various variant allele fractions (VAF) on sequencing reads from the NA12878 cell line sequenced with the TOMA OS-Seq 131 cancer gene panel with the Illumina platform. Here, we compared our method to a commonly used software, FreeBayes, showing better area-under-the curve when comparing ROC curves. We then validated our method by analyzing data from several reference materials with available ground truth, either engineered cell lines (Horizon Diagnostics: HD200, HD753, HD759), or spiked synthetic constructs (SeraCare, STMM Mix-II). Our algorithm could identify the 37 variants in the STMM Mix-II covered in our panel, including SNVs and indels, spiked at VAFs ranging from 5 to 40% with a sensitivity ranging from 94-97% and >99.99% specificity. **Conclusions:** To our knowledge our algorithm is the first method that includes score to explicitly differentiate somatic mutations from germline variants, it outperforms other callers and heuristic methods used in the field, and is particularly well suited for the clinical use cases where only the tumor specimen is available.

I03. Systematic Evaluation of Genetic Alterations for Cancer Precision Medicine Reports

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Introduction: The advent of NGS-based technologies for the detection of somatic mutations has brought about improvements/a rise in precision medicine - through new detection, management and treatment methods. Medical research has embraced the use of this technology in various aspects of clinical practice, especially in area of oncology which is at the forefront of these efforts. Numerous clinical trials are currently being conducted to exploit precision medicine for well-known genetic alterations. However, systematic evaluations of these genetic alterations for cancer-based precision medicine poses unique challenge due to complexities in bioinformatics and curation processes. **Methods:** Bioinformatics pipelines were created for processing of genetic alterations in each patient sample. This involved normalizing, annotating and mapping with manually curated databases. A curation interface was created to evaluate genetic alterations in association with two other components: disease and drug. In addition, several other parameters such as age, gender, race, stage and sensitivity of the mutations were considered in the process of creating this curated database. Clinical trials were seamlessly retrieved, free text automatically tagged by Natural Language Processing (NLP) for important entities, and presented on the curation interface for evaluations. Therapies and clinical trials based on the individual's genetic alterations were then further modified based on NCCN Guidelines®. **Results:** We have created a framework for the evaluation of these genetic alterations for cancer-based precision medicine and this is applied to each TheraKeyTM Clinical Interpretation Report. Through these interpretation results, the latest information on indicated therapies including FDA approved, Off-label and investigational drugs, contraindicated therapies (based on NCCN Guidelines®), and matched clinical trials (both worldwide and regional) that correspond to the patient's genetic variants can be easily obtained. **Conclusions:** Clinical interpretation of genetic alteration for cancer precision medicine requires systematic evaluation of several key parameters. These clinical interpretation results can effectively assist clinicians in finding clinical solutions for cancer patients, ultimately contributing to the improvement of each patient's treatment plan, survival rate and quality of life.

I04LB. Structural Variant Simulator Improves Variant Calling Accuracy in NGS

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Introduction: While structural variants are believed to account for at least 10% of pathogenic mutations, their detection is challenging. Consequently, it can be difficult to obtain sufficient clinical cases for development and validation. Instead, simulated variants created by synthesizing reads from a modified reference genome are often used. However, this approach does not account for key factors that affect structural variant detection, including sample quality, target enrichment chemistry, and platform-dependent artifacts. Here, we present a structural variant simulator that directly modifies aligned reads, resulting in a realistic noise model of both the capture and sequencing stages, and its application for evaluating structural variant calling methods. **Methods:** An *in-silico* simulator was developed, implementing 3 configurable types of structural variants: copy number variations (CNVs), insertions, and inversions. Changes in copy-number are achieved through a stochastic process of removing or duplicating reads in selected regions, while clipping reads that span breakpoints. Inversions and insertions are similarly simulated through modifying reads spanning breakpoints. Variants can be further modified by adding noise such as substitutions and indels. To evaluate the simulator, a high-homology region was selected; fewer than 10 clinical samples with known CNVs in the region were available for development. Parameters were tuned using a leave-one-out approach, but due to the small number of samples used for development, the caller was over-fitted. Further improvement was achieved by performing a literature review of CNVs in this region and simulating additional samples with CNVs using published breakpoints. The simulated samples increased the amount of data for development and validation significantly, enabling more robust parameter tuning and evaluation, resulting in 100% sensitivity. **Results:** The addition of realistic synthetic variants to a small number of biological specimens with CNVs in a high-homology region allowed the development of a caller with 100% sensitivity using a leave-one-out approach, while lower sensitivity was achieved without the synthetic samples, demonstrating the utility of realistic synthesis of structural variants for developing algorithms to call variants in regions where biological samples are rare. **Conclusions**: Simulating structural variants directly on aligned reads is an effective, realistic method for collecting data to develop structural variant detection algorithms. This approach adds power to validations for rare pathogenic mutations, and is publicly available at https://github.com/color/clrsvsim.

OTHER (Education, etc.)

OTH01. Application of the GeneReader NGS System in Analyzing a Diverse Set of Cancer Clinical Samples

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Introduction: One major goal of cancer clinical research is to establish new prognostic and predictive biomarkers for personalized diagnosis and therapy. Next-Generation Sequencing (NGS) has revolutionized the field of cancer genetic studies by dramatically decreasing costs and time needed for large-scale data generation and analysis. While NGS has proven to be a very useful tool in research, major hurdles remain for its broad adoption in the clinical research setting: lack of a seamless workflow, ability to handle samples from different sources or pre-analytical treatments, and lack of actionable content to guide the interpretation of results. Here, we apply a complete sample-to-insight NGS workflow, QIAGEN´s GeneReader System, to analyze a diverse set of clinical samples. **Methods:** Tissue samples from different tumor types (colon or melanoma) were subject to fixation by formalin or the PAXgene® system and embedded in paraffin. Donor-matched plasma samples were also analyzed when available. Sample DNA was processed and analyzed accordingly to the GeneReader Workflow: DNA was extracted using the GeneRead FFPE kit and the PreAnalytix PFPE Kit for PAXgene fixed tissue, respectively. Plasma DNA was isolated with the QIAamp® Circulating Nucleic Acid Kit. The quality was multiply assessed with the QIAxpert, the QIAxcel and the QuantiMIZE Kit. Targeted amplification of cancer hotspots was performed using the Actionable Insights Tumor Panel. After sequencing with the GeneReader, variants were identified using the QCI-Analyze software and interpreted with the QCI-Interpret software. **Results:** Despite different preanalytical treatments, all samples showed high quality scores in the QC steps. This was followed by successful amplification of target regions and all the subsequent workflow. For all samples (n=20) more than 99% of the sequence reads were mapped to the human genome (hg19) and > 90% to the regions of interest (ROI). The majority of the samples (19 out of 20) showed more than 95% of the ROI covered with at least 500 single reads. All variants were correctly identified and interpreted, showing the robustness of the GeneReader System and its flexibility and reliability handling different sample types and sources. **Conclusions:** In clinical research, the development of new prognostic and predictive biomarkers is often dependent on samples from biobanks and prospective cohorts which often suffer from suboptimal conditions such as long storage period and various treatment methods. The data presented show the advantages of a fully integrated sample-to-insight workflow including initial quality control steps and prove the versatility of the GeneReader System for different types of sample materials.
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SOLID TUMORS

ST01. HPV Detection and Genotyping in Formalin-fixed Paraffin Embedded Anal Lesions

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Introduction: Anal carcinomas are rare, with an incidence of only 1 to 2.5 cases per 100,000 people per year in many countries, and HPV is among the primary risk factors for the disease. HPV16 is the most prevalent type in anal carcinoma and is present in up to 89% of all patients with anal carcinoma. The objective of this study is to determine the frequency and subtype of HPV in anal lesions using formalin-fixed and paraffin embedded specimens (FFPE). **Methods:** Samples with anal carcinoma, high and low grade anal lesions were selected for HPV testing using the qualitative PCR Papillocheck® method (Greiner Bio-One). The PCR test interrogates 24 HPV subtypes (low risk: HPV6, 11, 40, 42, 43, 44/55; high risk: HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73 and 82). Some specimens were also analyzed by in situ hybridization (ISH) using the INFORM® HPV probes (Ventana) which detects 14 HPV subtypes (low risk HPV 6 e 11; high risk HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 66). **Results:** Eight-three samples were obtained for this study. HPV analysis was successful in 81 samples. Some cases failed in the PCR analysis (33.7%, n=28) but were successfully analyzed by ISH. The PCR failure is probably due to compromised DNA integrity related to tissue fixation in formalin. All the samples that failed in the PCR were originated from external laboratories. Two cases failed in both HPV detection methods and were excluded from further analysis. The majority of cases were carcinomas (86.5%, n=70), followed by low-grade (12.3%, n=10) and high-grade (1.2%, n=1) epithelial lesions. HPV was positive in 71.4% of carcinomas (50 out of 70), 90% of low grade (9 out of 10), and 100% of high grade lesions (1/1). HPV16 was the most prevalent subtype, detected in 44% (22/50) of the HPV positive carcinomas, followed HPV35 (3/50) and HPV45 (1/50). Additional 44% of cases (22/50) had high-risk (HPV 16, 18, 31, 33 or 35) but the subtype was not determined because these samples were assessed by ISH. **Conclusions:** HPV was detected in large frequency in anal lesions. Most of the HPV subtypes present in anal lesions were of high carcinogenicity. HPV16 was found in the majority of carcinomas. HPV frequency was also high in the high and low-grade lesions but number of samples in this group need to be expanded to allow further discussion. The PCR test Papillocheck® is useful to detect and genotype HPV in FFPE lesions but it may led to

inconclusive results in case with impaired DNA integrity. Despite the methodological limitation, ISH may complement the diagnosis in cases with inconclusive PCR results.

ST02. Automated Circulating Cell-Free Tumor DNA (cfDNA) Extraction and Preparation for qPCR and NGS

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Introduction: Circulating cell-free tumor DNA (cfDNA) has emerged as an important biomarker for non-invasive cancer monitoring. A large number of cancer genome sequencing studies in the US and worldwide have collectively identified the genetic changes that make human tumors grow and progress. In oncology, detection of cfDNA has also been considered as a potential prognostic marker for outcome in various cancers. One of the main challenges encountered in these methods relate to the efficient extraction of cfDNA from the "liquid biopsies". It is important to optimize the small amounts of highly fragmented tumor DNA from the blood. As assays for cfDNA are typically intended to identify genetic variants present at very low allelic frequencies, many of the established detection technologies are driven to the limits of their performance. The purpose of this study was to compare the performance of two cfDNA extraction systems: a column-based kit (QIAamp Circulating Nucleic Acid Kit) and an automated magnetic beads-based system (Sentosa® SX cfDNA Kit). **Methods:** We developed a magnetic bead-based cfDNA extraction kit, the Sentosa® SX cfDNA Kit and optimized it for use on the Vela Sentosa® SX101 platform. The Sentosa® SX101 is a CE-IVD certified robotic liquid handling system for nucleic acid extraction, PCR set-up and Next-Generation Sequencing (NGS) library preparation. We compared performance of the Sentosa® SX cfDNA Kit with a column-based cfDNA extraction kit (QIAamp). Integrity of cfDNA extracted by both methods was assessed using ALU repeats qPCR assay. Quality of the extracted cfDNA was tested using the NGS-based Sentosa® SQ CRC Panel. **Results:** In this study DNA was extracted from plasma samples spiked with 3 concentrations of fragmented HCT116 gDNA (KRAS G13D positive): 109, 54, and 27 mutant genome equivalents (GE) per 1mL of plasma using the Sentosa® SX cfDNA kit and a column-based cfDNA extraction kit, respectively. Fragment size of the cfDNA extracted by both methods was ~170 bp (confirmed by Bioanalyzer). The cfDNA portion in DNA samples extracted by the Sentosa® SX cfDNA kit was ~80% and for the column-based extraction method ~25%. **Conclusions:** The Sentosa® SX cfDNA Kit selectively extracts cfDNA in the presence of high molecular weight gDNA. The Sentosa® SX cfDNA Kit thus appears as an efficient and reliable solution for cfDNA extraction from human plasma samples. Integration into the Sentosa® qPCR- and NGS-based workflows makes the Sentosa® SX cfDNA Kit a comprehensive *in vitro* tool, which can be used in combination with various assays for detection of tumour derived cfDNA.

ST03. Targeted Bisulfite NGS of a DNA-methylation Multimarker Panel for Cancers of the Urinary Tract in Liquid Biopsies

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Introduction: In cancer treatment early detection is crucial for patients' prognoses and overall survival. Liquid biopsies (blood, urine, etc.) are of high interest as a tool of medical diagnostics for early cancer detection since sampling is easy, rapid and comparatively convenient for the patients. Putative tumor DNA usually occurs as a small fraction of the total of ubiquitous cell free DNA (cfDNA) circulating in the body. Such remnants of cell free circulating tumor DNA can be utilized for cancer diagnostics by measuring its divergent DNA methylation patterns as compared to normal background cfDNA. **Methods:** We established several single-plex PCR based assays to measure the methylation status of cancerous tissues via qPCR. Those biomarker assays enable us to reliably detect traces of tumor DNA from tissue from common cancers, as well as in cell free DNA (cfDNA) in urine and blood plasma samples from healthy subjects. The most informative tumor biomarker assays were multiplexed in a NGS prototype and used to analyze technical samples, clinical urine and blood plasma samples which were obtained from different commercial sources. cfDNA was extracted and bisulfite converted using the Epi proColon Plasma Quick Kit. The novel multiplexed biomarker panel was subsequently measured on bisulfite converted DNA via NGS on the Illumina platform in order to obtain information from 13 different tumor biomarkers in parallel for each patient.
Results: Methylation patterns of biomarkers from technical and clinical samples could be detected reliably and with high reproducibility via NGS measurements. Biological and technical replicates were highly correlated (>95%). In a first set of clinical samples we demonstrated that there is a clear pattern that enables to detect and distinguish cancers of the urinary tract. **Conclusions:** We established a prototype for a multiplex assay that can identify DNA-methylation patterns from cancers of the urinary tract using NGS. This prototype is a promising precursor and model for future diagnostic products and will need investigation in larger clinical studies to confirm its clinical value.

ST04. Multiplex detection of oncogenic fusion transcripts and MET exon skipping by molecular counting in lung cancer.

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Introduction: Genomic aberrations involving ALK, ROS1, RET and MET are druggable driver oncogenes causing expression of aberrant transcripts in lung adenocarcinomas. Given their relevance for targeted therapy, multiplex diagnostic assays are required for comprehensive detection in a clinical setting.
Methods: Clinical FFPE samples known to harbour ALK, ROS1, RET and MET aberrations, as well as some unclear and negative cases, were selected based on previously obtained Fluorescence In Situ Hybridization (FISH), Immunohistochemistry (IHC) and Next Generation Sequencing (NGS) results. Aberrant RNA transcripts were examined by a molecular counting assay based on direct multiplexed measurement of color-coded, target-specific barcodes. Both unique known fusions, as well as 3'/5' imbalance probes were measured. **Results:** The assay was able to accurately detect previously known fusions and MET exon skipping events, and even allowed to unambiguously score previous "unclear" FISH / IHC results. **Conclusions:** The applied molecular counting assay allows simultaneous detection of multiple targetable oncogene aberrations in FFPE lung cancer tissues. We believe that the established panel can complement, and in time potentially replace existing fusion assays for molecular diagnostics in lung cancer.

ST05. Head-to-Head Comparison of Two Commercially Available Next-Generation Sequencing Technologies that Detect Gene Fusions in Non-small Cell Lung Cancer

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Introduction: The reliable assessment of cancer-associated gene fusions by next-generation sequencing (NGS) is often challenged by low sample input quantity and quality, necessitating rigorous QC assessments to lend confidence to test results. Integration of these QC results with standardized reagents and bioinformatics is critical to assure consistent results from one laboratory to the next. Here we present a head-to-head comparison of two commercially available NGS kits that include reagents and software and are designed to detect non-small cell lung cancer (NSCLC)-related fusions. **Methods**: Total nucleic acid was isolated from 20 residual FFPE NSCLC biopsies and processed for NGS by an independent laboratory using the QuantideX® NGS RNA Lung Cancer Kit RUO (QDx) (Asuragen) and the FusionPlex® ALK, RET, ROS1 v2 Kit RUO (FP) (ArcherDx). RNA was quantified using the Qubit® RNA HS Assay Kit (Thermo Fisher). Sixteen FFPE samples were evaluated by each NGS kit using inputs ranging from 30-215 ng RNA. Four of these 16 samples were admixtures of known fusion-positive and negative FFPE RNA designed to comprise 2-15% variant (measured by functional copy number). An additional 8 FFPE samples were assessed by only pre-analytical QC using 20 ng RNA, corresponding to the minimum stated input requirements for both kits. Libraries were sequenced on the MiSeq® System (Illumina), and analyzed using each kit's bioinformatics software suite. **Results**: Of the 16 samples evaluated by NGS, all passed each kit's pre-analytical QC criteria and were processed into NGS libraries. All libraries also passed post-NGS QC criteria for QDx and generated the expected fusion calls. However, only 50% of the libraries created by the FP kit passed post-NGS QC metrics. Specifically, 6/9 libraries with ≤70 ng input failed this QC along with two libraries with 150 ng input. In contrast, 5/7 libraries with ≥150 ng input passed as well as three libraries with 38-70 ng input. False negatives occurred in 5/8 failed samples for FP, underscoring the value of post-NGS QC. Pre-analytical QC results for FFPE input at 20 ng were consistent with other low-input samples processed through NGS for FP and QDx. **Conclusions**: Both kits included reagents from library prep to bioinformatics and integrated multiple QC metrics. Both kits also generated accurate calls when pre- and post-analytical QC criteria were satisfied. QDx accommodated at least 5-fold lower RNA inputs and a higher rate of FFPE samples passing QC than FP. FP, however, can identify novel fusions that would require 3'/5' expression imbalances to flag using QDx. Our results emphasize the importance of verifying minimum input requirements for each NGS technology and scrutinizing QC checkpoints to ensure reliable results.

ST06. Robust Mutation Profiles in Cancer Biopsies and Resections

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Introduction: Sequencing technology has recently been improved to reduce the impact of *in vitro* sequencing error and to improve accuracy of measured variant allele fractions. We tested this new strategy by applying commercial library preparation reagents that 1) tag "unique molecular identifiers" to the ends of natural DNA fragments so that PCR replicates are traceable to their original template, 2) amplify to the end of each fragment so that varying fragment lengths further contribute uniqueness not achievable with standard amplicon sequencing, and 3) sequence both strands to confirm variant calls. **Methods:** For each of 30 gastric adenocarcinoma biopsy (n=10) or resection (n=20) specimens, macrodissected paraffin embedded tissue was sequenced across hotspots in 67 human cancer genes (Archer VariantPlex Solid Tumor Panel library reagents; Illumina MiSeq with v2 or v3 sequencing reagents; Archer Analysis software v.4.1.1). Three cancer biopsies and a normal

control DNA underwent replicate library preparations and/or sequencing runs. Non-synonymous mutations and small indels at allele frequency >5% were cataloged. **Results**: Two resected cancers could not be sequenced due to insufficient library quantity. The remaining 28 cancers harbored multiple non-synonymous gene variants, many of which are in genes previously associated with gastric cancer. Average read count per tumor was 1.4 million (minimum 486k), and average % reads that were unique was 30% (minimum 8%). There were ~119 unique fragment lengths per primer set. Average unique depth of coverage was 464x (minimum 119x). Compared to resections, biopsies had 17% fewer unique reads, on average. Replicate libraries or sequencing runs yielded equivalent variant calls and variant allele frequencies. **Conclusions**: Successful sequencing of both small biopsy and resection specimens is achievable using "unique molecular identifiers" and varying amplicon lengths to demonstrate the extent to which diverse natural molecules are represented in the assay. Replicate test results demonstrate good analytic reproducibility, suggesting that the software algorithm minimizes the impact of *in vitro* sequencing errors. Dual strand sequencing bolsters confidence that variant calls are legitimate. In summary, enhanced massive parallel sequencing technology is robust for mutation detection and quantification even in small, fixed biopsy specimens.

ST07. Plasma Mutation Profile Reflects Active Cancer Status

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Introduction: Cancer patients often have high amounts of plasma DNA derived from apoptotic or necrotic cells, exosomes, and other microvesicles. Modern sequencing technology facilitates detection of cancer-specific mutations in plasma DNA that could reflect active tumor status. **Methods:** Total nucleic acid was extracted from 16 plasma specimens from 16 active cancer patients. Each cancer had known mutations based on previous massive parallel sequencing of tissue representing melanoma; adenocarcinoma of lung, colon or pancreas; or head and neck squamous cell carcinoma. Nucleic acid from 1mL of EDTA plasma was sequenced across hotspots in 28 human cancer genes (Archer Reveal ctDNA 28 library reagents; Illumina NextSeq with Mid v2 sequencing reagents; Archer Analysis software v5.0.0). Non-synonymous mutations and small indels at allele frequency >.3% were cataloged. **Results:** All 16 plasmas yielded robust sequencing data. At least one known tumor-related mutation was detectable in 15/16 active cancer patient plasmas at variant allele fractions from <1% to 73% (average 14%). Average read count per plasma was 4.6 million (minimum 3.3 million), and average % reads that were unique was 16% (minimum 6%). There were ~226 unique fragment lengths per primer set. Average unique depth of coverage was 2827x (minimum 653x). One lung cancer patient had an *EGFR* T790M mutation in plasma that was not present in initial diagnosis cancer tissue, implying outgrowth of a clone having an acquired drug resistance mutation. Additional mutations found in plasma but not in cancer tissue were generally at allele fractions below 1%. **Conclusions:** Our findings demonstrate the potential for non-invasive detection of tumor-related mutations to facilitate tracking tumor burden and to characterize emerging clones. Features of this test system include 1) off-the-shelf reagents and software promotes inter-laboratory comparisons, 2) both strands are sequenced to confirm variant calls, 3) unique molecular identifiers as well as variable amplicon lengths help quantify natural templates and also assure adequate diversity of DNA that was sequenced, and 4) applicability to a wide range of cancer patients since common oncogene hotspots and all *TP53* exons are sequenced.

ST08. Cancer of Unknown Primary Site: The Critical Role of the Genomics Profile and Public cBioPortal

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Introduction: Cancer of unknown primary site is comprising 3% to 5% of all malignancies and is a major challenge in surgical pathology. Pathologic diagnosis is achieved by subjecting surgical specimens to immunohistochemistry and more recently gene expression profiling has been used to assist in determining the most likely tissue of origin. The Cancer Genome Atlas (TCGA) and other comprehensive genomic profiling initiatives have also provided insight into patterns of mutational profiles and copy number abnormalities associated with specific tumor types. Public data mining tools like cBioPortal have facilitated analysis and visualization of these large-scale cancer genomics datasets. **Methods**: A 60-year-old white woman presented with metastatic carcinoma to the left humorous and left ilium with unknown primary in 2014. She was on systemic chemotherapy and palliative course of radiation treatment with unclear response. PET-CT showed progression of metastatic disease of left proximal humorous and new hypermetabolic left axillary adenopathy in May 2016. Left deep axillary lymph node was resected, positive for poorly differentiated adenocarcinoma. The lymph node tissue was submitted for genomic profile to the Foundation one. **Results**: Three genomic alterations were identified: a frame shift mutation of *NF2* gene (*NF2* K44fs*79), a nonsense mutation of *ARID2* gene (W1636*), and a missense mutation of *TP53* K139N. This combination of genomic alteration was queried in the public cBioPortal and the result was suggestive of renal origin. The case was presented in monthly Emory Molecular Tumor Board and the lesion was worked up extensively in an attempt to identify a primary source. The immunohistochemical profile was also suggestive of renal

origin. Further imaging studies identified a right kidney lesion which was not detected on the PET-CT scan. **Conclusions**: This case represents a diagnostic challenge for pathologists. Comparing the genomics profile of this patient's tumor with public datasets available in cBioPortal guided us to the probable origin of the metastatic tumor, which could be confirmed with better informed immunohistochemistry studies. The Molecular Tumor Board was a critical component of this process and is an important part of the precision medicine in all hospitals.

ST09. Circulating Tumor Cells Detection Using hTERT and MAGE A1-6 PCR and Conditionally Reprogrammed Cell Culture

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Introduction: To look in cancer cell circulation, much studies have been performed and developed into the liquid biopsy. However, detection of circulating tumor cells (CTC) was still unresolved. We tried to detect CTCs using cancer specific PCR and the conditionally reprogrammed cell (CRC) technology. The CRC technology uses a co-culture system of tumor cells with irradiated 3T3 fibroblast feeder cells (J2 cells) and the Rho kinase inhibitor Y-27632. **Methods:** We confirmed the sensitivity of CRC technique using cancer cells. Two sets of 3mL blood were obtained from the 96 cancer patients composed of 56 colorectal cancers, 25 gastric cancers and 15 breast cancers. To collect CTC from the blood specimens, we lysed RBCs using Roche lysis buffer (Roche, Basel, Switzerland), the blood mononuclear cells were collected. The first blood set were treated with 1mL of Trizol, then hTERT, MAGE A1-6 and GAPD genes were amplified to detect the tumor cells. For the second blood set, we cultured the collected cells at 37°C with CRC technique for 4 weeks. At the end of incubation, the culture plates were treated with 1mL of Trizol, then hTERT, MAGE A1-6, CEA (for colorectal and gastric cancer), mammaglobin (for breast cancer) and GAPD genes were amplified to confirm the tumor cells. We defined the grown cells as cancer cells if more than 2 cancer genes were expressed. **Results:** The lowest limit of CRC technology for CTC detection was 6.3 cells for gastric cancer cells. From the blood of cancer patients, hTERT and MAGE PCR showed 40.6% and 15.6% positive rates respectively. In the CRC culture, the hTERT and MAGE PCR disclosed 17.7% and 5.2% positive rates. Among cultured cells, 8 cultured cells were confirmed as cancer cells: 5 colorectal, 2 gastric and 1 breast cancer. The culture hTERT PCR showed statistically significant correlation with cancer stage and blood hTERT PCR. **Conclusions:** The CTC were cultured with the CRC technology. Though the sensitivity of CRC technique was not high, it would be useful to understand CTC. The blood hTERT or MAGE A1-6 PCR showed the most sensitive result while the culture hTERT PCR was related with cancer stage.

ST10. WITHDRAWN

ST11. Detect 0.1% Low Frequency Somatic Variants in Cell-free DNA Using Oncomine™ cfDNA Assays and Ion Torrent Sequencing

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Introduction: Study of genetic Information from cell-free (cf) DNA provides valuable opportunities in cancer research with potential future impact to the field of oncology. As an example, liquid biopsy provides a non-invasive and cost effective solution compared to traditional solid tissue biopsy tests. Here we report the application of the research based Ion Torrent™ next-generation sequencing (NGS) Oncomine™ cfDNA assays and associated workflow, which has been developed to detect somatic variants at low frequency of 0.1% minor allelic fraction in cfDNA from plasma. **Methods**: cfDNA was isolated from plasma of Non-small Cell Lung Cancer (NSCLC) banked research samples (MagMAX™ Cell-Free DNA Isolation Kit, Thermo Fisher Scientific), and their matched tumor DNA was extracted from Formalin-fixed, Paraffin-embedded (FFPE) tissue (RecoverAll™ Multi-Sample RNA/DNA Isolation kit, Thermo Fisher Scientific). Additionally cfDNA Reference Standards (Horizon Discovery) were used to evaluate assay sensitivity and specificity against known engineered variants. Isolated cfDNA and FFPE DNA was "molecularly" barcoded with a set of gene specific primers followed by library amplification. Libraries were then purified with Agencourt Ampure XP™ beads (Beckman Coulter) and quantified with qPCR (Ion Library Quantitation Kit, Thermo Fisher Scientific). Multiplexed libraries were templated with Ion Chef automated templating instrument (Thermo Fisher Scientific) and sequenced with Ion S5 Sequencing instrument (Thermo Fisher Scientific). Data were analyzed using Torrent Suite Software (TSS). **Results**: Oncomine™ cfDNA Assays generate targeted libraries for sequencing with the Ion Torrent™ NGS technology and tailored TSS analytical pipeline allowing detection of variants as low as 0.1% limit of detection (LOD) from 20ng of cfDNA. The total process time (from plasma/FFPE specimen to result reporting) is 32 hours with a total hands on time of 4 hours. Using cfDNA Reference Standards (0, 0.1, 1.0 and 5.0%), 8 known variants were detected with 89.6% sensitivity and 99.8% specificity at 0.1% LOD, 100% (sensitivity and specificity) at both 1.0% and 5.0% LOD. When testing cancer research samples with matched FFPE and plasma, somatic variants were detected at a frequency between 0.28 % and 4.32 % in cfDNA and between 6.85 % and 74.04% in corresponding DNA isolated from FFPE. **Conclusions**: The Oncomine™ cfDNA Assays provide an easy, quick and reliable solution

for research in detecting low frequency somatic variant in blood plasma and tissue. Results from this study support further testing of these and similar sequencing methods for liquid biopsy for future applications in cancer diagnosis and disease monitoring.

ST12. A Novel Fusion of TFE-3 and LINC01010 in Renal Cell Carcinoma

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Introduction: The Xp11 translocation Renal Cell Carcinomas (RCC) are characterized by gene fusions involving *TFE3*. The result is a *TFE3* transcription factor gene fusion with one of multiple reported genes including i*ASPL* (*ASPS*), *PRCC*, *SFPQ* (*PSF*), *NonO* (*p54*), and *CLTC*. Break-apart TFE3 fluorescence in situ hybridization (FISH) on formalin-fixed paraffin-embedded (FFPE) tissue sections is currently the gold standard for identification of TFE3 rearrangements. Recently, next-generation sequencing (NGS) techniques at the transcriptome level (RNA-Seq) have been used to verify known and discover novel transcribed gene fusions. **Methods**: A 57-year old male patient with metastatic RCC, diagnosed in April, 2015 underwent left nephrectomy, adrenalectomy, and lymphadenectomy. In June 2015, follow-up imaging study showed development of metastatic disease involving retroperitoneum, pelvis and liver. The disease progressed despite multiple lines of chemotherapy. The Anchored Multiplex polymerase chain reaction (AMP) assay and sequencing was done on the nephrectomy specimen. **Results:** The FISH test was negative for TFE3 translocation, however, the NGS assay was able to show strong evidence of TFE3-LINC01010 fusion, t(X;6)(p111;q23). The LINC01010, long intergenic non-protein coding RNA1010 is a novel fusion partner gene of *TFE3*. There is a percentage of 27.5 % in reference to the total reads covering this gene fusion, including wild-type transcripts (the tumor cellularity ~ 20-30%) This gene fusion results in overexpression of the TFE3 RNA and protein (confirmed by immunohistochemistry). The result of the confirmatory RT-PCR is pending. **Conclusions:** The NGS based fusion gene assay is a robust technique to detect gene rearrangements from FFPE material. This assay is able to identify the novel fusion gene products which may be the target of future therapy. More investigation is needed to better understand the mechanism of TFE3 overexpression.

ST13. Spectrum of RAS Mutations in 946 Korean Patients with Metastatic Colorectal Cancer

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Introduction: Current metastatic colorectal cancer therapy uses anti-epidermal growth factor receptor (EGFR) monoclonal antibodies. Metastatic colorectal cancer harbouring *RAS* mutations dose not benefit from therapy with anti-EGFR therapy. All patients with metastatic colorectal cancer should have tumor tissue genotyped for *RAS* mutations (*KRAS* and *NRAS*). The aim of this study is to determine the frequency and type of the *RAS* mutations in Korean patients with metastatic colorectal cancer. **Methods:** PCR amplification and direct sequencing of *KRAS* gene (exon 2, 3 and 4) and *NRAS* gene (exon 2, 3 and 4) were performed in 946 patients with metastatic colorectal cancer, classified as statge IV according to TNM system. Genomic DNA was extracted from hematoxylin/eosin-stained sections (5 um thickness) of representative paraffin –embedded blocks using QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). Bidirectional sequencing was performed using the BigDye Terminator v1.1 kit (Applied Biosystems, Foster City, CA, USA). **Results:** We detected the mutations in 329(34.8%) of 946 patients with metastatic colorectal cancer by direct sequencing: 32.7% (309/946) for *KRAS*, 2.1% (20/946) for *NRAS*. The most prevalent mutation was c.35G>A (p.Gly12Asp) in *KRAS* accounting for 39.2% (129/329) of all detected mutations, followed by c.38G>A (p.Gly13Ala) (17.8%, 58/329) and c.35G>T (p.Gly35Val) (14.7%). Among the 309 *KRAS* mutations, frequency of codons 12 and 13 was 68.4% and 19.0%, respectively. The most frequent mutation in *NRAS* was c.35G>A (p.Gly12Asp) accounting for 35.3% (7/20) of all detected NRAS mutations. The mutation frequency of non-exon 2 *KRAS* (exon 3 and 4 of *KRAS*) and *NRAS* was 6.2% (59/946). Especially, c.436G>A (p.A146T) was detected in 7.0% frequency (23/329). Concomittent mutation of *KRAS* and NRAS was not detected. **Conclusions:** These data provide information about spectrum of KRAS and NRAS and have important implications for mutation testing for Korean patients with metastatic colorectal cancer. The present study is the first to provide data on the frequency and types of non-exon 2 *KRAS* and *NRAS* for unselected Korean colorectal cancer patients tested in a routine setting.

ST14. A Novel AKAP13-NTRK3 Fusion in a Radiation-Induced Sarcoma of the Central Nervous System

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Introduction: Radiation-induced sarcomas (RISs) of the central nervous system are an uncommon late risk of irradiation with an estimate rate of 0.03-0.3% of patients who have undergone radiation therapy. RISs are often high-grade tumors with poor prognosis. Recently, next-generation sequencing (NGS) techniques at the transcriptome level (RNA-Seq) have been used to verify known and discover novel transcribed gene fusions.

Methods: A 32-year old male patient had a previous history of primitive neuroectodermal tumor (PNET) treated with total resection in 1993 and followed by 4 rounds of chemotherapy and 6 weeks of radiation. A suprasellar mass was discovered in April 2014. The tumor biopsy showed bland epithelioid to spindle cells embedded in a myxohyaline matrix with extensive involvement of adjacent tissues. The microarray study and the Anchored Multiplex polymerase chain reaction (AMP) assay and sequencing were done. **Results:** We detected a novel AKAP13-NTRK3 fusion. These two genes both are located on Chromosome band of 15q25.3, and are 2.20Mb apart with opposite orientation. The *AKAP13* is closer to centromere in a sense direction and *NTRK3* in an antisense direction. Also, the RNA expression showed overexpression of NTRK3 compared to VCP control gene. The copy number analysis was also performed and multiple complex abnormalities including: gain of 7p, gain of 15q encompassing the region of *NTRK3* and *AKAP13* and beyond, loss of 11 containing the *MEN1* gene, and loss of 13q containing the *RB1* gene. The result of the confirmatory RT-PCR is pending. **Conclusions:** The RNA-based NGS fusion gene assays are a robust technique to detect gene rearrangements from FFPE material. These assays can identify the novel fusion gene products which may be the target of current or future therapies. Currently, there are multiple chemotherapies targeting solid tumors that harbor activating alterations to NTRK3 which may be a therapeutic option for this patient.

ST15. Defining the Molecular Profile in Patients with Non-small Cell Lung Carcinoma (NSCLC) and its Relation to First-line Treatment in a Comprehensive Cancer Center in Mexico City

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Introduction: Molecular characterization of lung NSCLC is of relevance for the identification of patients that may benefit from targeted therapies. Genetic drivers such as EGFR, KRAS and ALK are now routinely determined, and represent 30-40% of all mutations. Scarce data exist on Mexican population. The aim of the study is to describe the clinicopathological characteristics and molecular profile of NSCLC patients studied in our laboratory and correlate the treatment options with their survival outcome. **Methods**: We reviewed cases diagnosed with NSCLC from 2012-2015 at the department of molecular pathology of our institution. Ninety cases were retrieved, 77 adenocarcinoma, 6 squamous cell carcinoma, 3 large cell carcinoma and 4 mixed cells types. DNA extraction was performed with QIAamp FFPE Kit (Qiagen).EGFR and KRAS mutations were determined with the Scorpion-ARMS technique (Therascreen/Qiagen). ALK rearrangement status was determined by FISH (ALK-LSI probes-Abbott Molecular), and evaluated using an OlympusBX53 microscope. Mean and standard deviation were determined, the results compared using T-Student test. Concordance of clinical stage, histologic subtype and treatment according to NCCN/ESMO guidelines was evaluated and correlated with outcome, follow-up information was analyzed using Kaplan-Meir curves and Cox regression model. **Results**: Histologic subtyping according to IASLC classification showed: predominant acinar pattern in 56%, solid 20%, lepidic 15%, and 9% micropapillary pattern. The most frequent clinical stage was IV (53%) followed by I (18%), II and III (14%). Within these different clinical stages, the initial treatment included surgery (43.8%), combination radiotherapy (15.6%) and chemotherapy (67%). The frequency of mutation for KRAS (21%), EGFR (18%) and ALK (1%) did not show significant differences between age or clinical stage. Follow-up and treatment data were available in 64 cases. Global mortality during the study was 47.5%, with a death rate of 0.18(IC 95% 0.11 - 0.028). Overall survival at 18 and 24 months was 0.58(IC95% 0.41-0.71) and 0.42 (0.24-0.60). First-line treatment according to mutation status was concordant in 86.5% and concordant with clinical staging in 83%. Multivariate survival analyzes showed that discordant treatment is an independent prognostic factor of mortality with 18 vs 41 months, ($p<0.001$) HR = 2.021(IC95% 1.9 – 1.66). **Conclusions**: KRAS is the most frequent mutation and mutually exclusive with EGFR and ALK. EGFR mutation had a higher frequency in female patients. Triple negative cases represent more than half of our NSCLC patients, posing great need to explore other targetable genetic drivers. Adherence to standard treatment guidelines is of mayor importance to gain the maximum survival benefit for our patients.

ST16. C1GALT1 Promotes EGFR Activity and is a Potential Therapeutic Target for Head and Neck Squamous Cell Carcinoma

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Introduction: Head and neck squamous carcinoma (HNSCC) consists of squamous carcinoma arising in the oral cavity, oropharynx, hypopharynx, and larynx. Despite multidisciplinary treatments, the five-year overall survival rate is still about 40-50%. Hence, identifying new therapeutic targets is urgently needed for HNSCC. Glycosylation is the most common post-translational modification of proteins, and aberrant glycosylation is a hallmark of cancers. Core 1 β1,3-galactosyltransferase (C1GALT1) transfers galactose (Gal) to Tn antigen (GalNAcα1-Ser/Thr) forming the T antigen (Galβ1-

3GalNAcα1-Ser/Thr). Both Tn and T antigens, short O-glycans, have been found in HNSCC. C1GALT1 is overexpressed in several cancers and promotes their malignant phenotypes. However, the expression and function of C1GALT1 in HNSCC remain unclear. **Methods:** Immunohistochemistry was performed to analyze expression of C1GALT1 in 117 HNSCC tumors. Student *t*-test and Kaplan-Meier analysis were used to analyze correlation of C1GLAT1 expression with clinicopathological factors and survivals. MTT assay, transwell migration, and Matrigel invasion assays were carried out to evaluate cell viability, migration, and invasion, respectively. Human phospho-RTK array and Western blot analyses were performed to evaluate signaling pathways. ELISA was used to evaluate EGF-EGFR binding affinity. Molecular docking simulation was used for searching C1GALT1 inhibitors. *In vivo* effect of C1GALT1 and its inhibitor was evaluated in NOD/SCID mice. **Results:** C1GALT1 was overexpressed in HNSCC tumors compared with non-tumor mucosa. Higher expression of C1GALT1 was associated with poorer disease-free survival and overall survival (n = 117). Moreover, C1GALT1 overexpression enhanced whereas C1GALT1 knockdown suppressed cell viability, migration, and invasion in HNSCC cells. Mechanistically, C1GALT1 modulated O-glycosylation of EGFR and enhanced EGF-EGFR binding affinity, leading to increased EGFR signaling. Moreover, we identified itraconazole as a potential C1GALT1 inhibitor, which directly bound to C1GALT1 and changed O-glycans on cell surfaces and EGFR. Silencing of C1GALT1 with shRNA or itraconazole was able to significantly suppress tumor growth in NOD/SCID mice. **Conclusions:** Our findings indicate that C1GALT1 promotes malignant phenotypes of HNSCC cells and is a potential therapeutic target for HNSCC.

ST17. Correlating MDM2 and CDK4 Amplification Levels in Dedifferentiated Liposarcoma (DDLS) with Tumor Behavior and Patient Outcome

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Introduction: Liposarcoma with *MDM2* gene amplification is one of the most common subtypes of adult sarcoma; however, other than the presence of dedifferentiation and FNCLCC grade, molecular prognostic markers are limited. Genetic abnormalities underlying the pathogenesis and behavior of DDLS are not fully understood. The aim of this study is to evaluate the correlation of specific CNAs with DDLS behavior and patient outcome to identify prognostic markers for risk stratification of liposarcoma patients. **Methods:** FFPE tumor tissues from 47 DDLS with clinical and pathological information were obtained for DNA extraction and cytogenomic microarray analysis (CMA). The amplification levels of *MDM2* and *CDK4* were also evaluated with droplet digital PCR analysis and correlated with the results obtained from CMA analysis. Patient outcome data include decreased disease free survival (DFS) and disease specific survival (DSS) retrieved from our institutional electronic medical records. Gene copy numbers for 12 loci were evaluated and correlated with outcome data using cut point analysis and comparison of Kaplan-Meier survival curves by log rank tests. **Results:** The results show that copy number alterations including chromosomal gains, amplifications, and losses were very common in DDLS. Amplification of genes (defined as 5 or more copies) within the 12q13-15 region were most common as previously described. Statistical analysis showed that DDLS with high levels of *MDM2* (> 38 copies) and *CDK4* (>30 copies) gene amplification showed decreased DFS (p = 0.0168 and 0.0169 respectively) and DSS (p = 0.0082 and 0.0140 respectively). *MDM2* and *CDK4* also showed evidence of an interactive effect so that each additional copy of one enhances the effect on prognosis of each additional copy of the other. In addition, higher levels of *MDM2* amplification were also significantly more likely to be found in retroperitoneal tumors compared to all other sites. **Conclusions:** We have shown that the levels of both *MDM2* and *CDK4* amplification in DDLS have prognostic value. Furthermore, the amplified genes may display an interactive effect whereby as the amplification level of one gene is increased the effect on prognosis of each additional copy number of the other gene becomes greater. This interactive effect is best demonstrated in the Cox proportional hazard model. Further studies are needed to confirm this finding. With the promise of targeted therapies directed towards *MDM2* and *CDK4*, determining whether amplification levels of these genes could predict response to these drugs may represent an area of interest for future investigation. Correlations of other copy number alterations with DDLS tumor behavior and prognosis will also be reported and discussed.

ST18. MUC20 Knockdown Suppresses Malignant Phenotypes of Pancreatic ductal Adenocarcinoma Cells Induced by Pancreatic Stellate Cells

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Introduction: Pancreatic Ductal Adenocarcinoma (PDAC) is usually surrounded by a dense fibrotic stroma consisting of extracellular matrix and pancreatic stellate cells (PSCs). The stroma creates hypoxic and nutrient-deprived microenvironment and promotes the malignant behavior of PDAC cells. Mucins have been reported to play a critical role in the pathogenesis of PDAC and regulate PSC functions. However, the role of mucin (MUC) 20 in PDAC remains unclear. **Methods:** Immunohistochemistry was performed to detect MUC20 in paraffin-embedded tumor tissues of PDAC patients (n=150). Real time RT-PCR and Western blot analyses were used to determine the expression level of MUC20 in PDAC cells. MUC20 was

knocked down with siRNA or shRNA. PDAC cellular properties were analyzed by MTT, transwell migration, and Matrigel invasion assays. Tumor growth in vivo was assessed by intraperitoneal injection of PDAC cells in nude mice. **Results:** The Oncomine database indicated that MUC20 mRNA was frequently overexpressed in primary PDAC tumors compared with normal pancreas. High MUC20 expression correlated with poor survival of PDAC patients. MUC20 knockdown inhibited PDAC cell viability, migration, and invasion induced by conditioned medium of PSCs. MUC20 knockdown decreased tumor growth in mice. Interestingly, the expression of MUC20 was induced by hypoxia and serum starvation in PDAC cells. We further found that HGF-MET signaling pathway was suppressed by MUC20 knockdown in PDAC cells.
Conclusions: MUC20 is associated with poor survival of PDAC patients and enhances PSC-mediated malignant phenotypes in PDAC cells, suggesting MUC20 as a potential therapeutic target.

ST19. Detection and Classification of Clinical Tumor Variants Using the QIAGEN GeneReader NGS System
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Introduction: The identification of actionable mutations has resulted in novel treatments and improved outcomes for various solid and hematological malignancies. While the use of Next-Generation Sequencing (NGS) in the clinical laboratory has continued to expand, there is a growing need for integrated wet bench and analytic pipelines. The Qiagen GeneReader NGS System and the Qiagen Actionable Tumor Panel (ATP) has been designed as such, from semi-automated FFPE extraction to integrated bioinformatics and clinical insight, potentially allowing for wider clinical adoption of NGS solid tumor sequencing. Here, we compared the Qiagen GeneReader System (ATP) with our current clinical pipeline using the IonTorrent PGM (Ampliseq Tumor Hotspot Panelv2). **Methods:** Fifty (31 unique, 19 duplicate) FFPE samples from lung, colon, melanoma, and GIST tumors were extracted, sequenced, and analyzed on the IonTorrent PGM and the Qiagen GR system. The clinical pipeline for the Ampliseq Cancer Hotspot Panelv2 (50-gene) has been published and includes 500X coverage for 10 samples on a 318 Chip with a custom GoldenHelix bioinformatics pipeline. Sample preparation and sequencing on the GeneReader NGS system was performed according to the manufacturer's instructions using the hotspot Actionable Tumor Panel (12-gene) and 10 samples/flowcell. Bioinformatics and clinical interpretation were performed using the included Qiagen Clinical Insight-Analyze (QCI-A) software package and Qiagen Clinical Insight-Interpret (QCI-I) online service. **Results:** The stock analysis for the Qiagen panel focuses on the detection of 773 unique variant positions in 12 genes. While additional bases are sequenced, this comparison was limited to clinically actionable variants. Of the 50 samples only one failed to meet established QCI-A quality metrics and was removed from further analysis. We noted high concordance (100%, 30/30 unique samples) across the actionable variants reported by QCI-I in comparison to those identified on the PGM. We also noted high reproducibility of the Qiagen GR System within duplicate samples (19/19). Greater than 99% of variant positions were covered at over 500X with KRAS p.A146 covered at >200 but <500X in 12 samples across 4 of 5 flowcells. **Conclusions:** The Qiagen GeneReader System is a fully-integrated NGS platform that offers the ability to identify a number of clinically relevant variants for clinical molecular oncology testing. The inclusion of a bioinformatics and clinical interpretation pipeline provides the user with up-to-date clinical information required in making clinical observations without the need for establishing additional in-house knowledge bases and maintaining additional analytics software packages.

ST20. Application of Liquid Biopsy to Uncover Critical Cancer Insights Using the GeneReader NGS System
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Introduction: Next-Generation Sequencing (NGS) is a powerful tool for the molecular characterization of tumor, and is used extensively in cancer research. These efforts are primarily based on studies of formalin-fixed paraffin-embedded (FFPE) samples, the use of which is limited by surgical feasibility, tissue availability and patient preference. An emerging field of study is focused on circulating-free DNA (cfDNA), sometimes termed "Liquid Biopsy". These DNA fragments are released by the tumor mass into the circulatory system, and their mutational status can often shed light on disease characteristics. While holding great promise as the future of cancer understanding and management, the liquid biopsy field is still nascent, lacking an integrated NGS workflow solution with proven performance. **Methods:** QIAGEN is an established expert in the field of sample preparation for liquid biopsy. The combination of the QIAamp Circulating Nucleic Acid Kit, the GeneReader NGS System using a new proprietary sequencing chemistry, and an optimized bioinformatics solution, provides the first fully integrated workflow specifically designed for analyzing cfDNA. A set of liquid biopsy samples from non-small cell lung cancer (NSCLC) patients and cfDNA reference standards (Horizon), with allelic frequency down to 1%, were used for this study. cfDNA was extracted from the liquid biopsy samples, and run through the QIAGEN GeneReader workflow. Variants were analysed using the QIAGEN Clinical Insight (QCI™) Analyze for liquid biopsy. **Results:** Expected mutations in all genes (EGFR, KRAS, NRAS,

PI3KCA) were identified and verified, at 100% concordance with reported in ddPCR studies. Both single nucleotide and insertion-deletion variants were correctly detected at down to a 1% allele frequency threshold. When tested through repeat runs, the results were consistent with an average Standard Deviation (SD) of 0.1%. No significant difference was observed between cfDNA reference standards and clinical liquid biopsy samples. These data exceed the 'Analytical Performance Specifications for Somatic Variant Detection Using Circulating Tumor DNA' as specified by the Palmetto GBA (M00135, V2, 2016). **Conclusions:** These data exhibit high sensitivity and performance consistency of liquid biopsy analysis with the GeneReader system. This is the first study of its kind to systematically demonstrate the accuracy of a cfDNA assay combined with the robustness of a fully integrated NGS system. As such, laboratories interested in conducting liquid biopsy studies can leverage this study and the system to implement an NGS workflow.
Disclaimer: some of the data in this study was obtained using a sequencing chemistry only available outside of the United States.

ST21. Using GeneReader NGS System to Identify Germline and Somatic Mutations in BRCA 1/2 Genes
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Introduction: BRCA 1/2 genes likely represent the best examples of modern understanding of cancer molecular genetics. Supported by strong clinical evidence and popularized by celebrity incidences (e.g. Angelina Jolie), testing for germline mutations in BRCA 1/2 in suspected familial breast cancer cases has gradually become common practice, especially in countries like the US. However, while less widely known and much harder to detect, somatic aberrations in these genes also play a critical role in cancer evolution, outcome and management. Their importance is further underscored by the development and launch of a new class of PARP antagonists such as Olaparib. **Methods:** In order to enable full investigation of mutations in BRCA 1/2 genes, especially in somatic settings, we sought to develop an NGS-based assay compatible with a range of clinical samples. To provide a fully integrated solution enabling any laboratory to adopt the test, we used the GeneReader NGS System, combining wet bench sample processing, target sequence enrichment, and optimized bioinformatics algorithms for specific variant calling and interpretation for BRCA genes. We then tested the performance of this assay by running a set of clinical samples harboring BRCA 1/2 mutations with previously confirmed allele frequencies. **Results:** The assay was designed to cover the entire coding regions of both genes, with 20bp flanking sequences into intronic regions. It was also designed to detect both Single Nucleotide Variants (SNVs) as well as Insertion/Deletions (InDels) of different sizes. A bioinformatics pipeline was also developed to specifically support variant calling in this assay. Greater than 30 clinical samples were pooled and run in a 6-plex configuration per flowcell, and yielded sequencing results with an average coverage of 5000 across all target regions. Greater than 99% of the target regions showed >500x (99.9% at >200x), demonstrating sufficient and uniform amplification and sequencing coverage, with mutant alleles detected at down to 5% allele frequency. Mutational status was correctly confirmed in all samples, resulting in 100% concordance with an alternative testing technology. **Conclusions:** These results support the use of the GeneReader System to detect BRCA 1/2 mutations relevant to both germline and somatic cancers. This fully integrated workflow together with an optimized BRCA pipeline enables an easy implementation of the test by any molecular cancer research laboratory.

ST22. Frequency of EGFR Mutations Among Multiethnic Filipino Patients in a Tertiary Care Setting
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Introduction: Epidermal Growth Factor Receptor (EGFR) mutation status has a significant prognostic and predictive role in the management of Non-Small Cell Lung Cancer (NSCLC). Although EGFR mutation testing in NSCLC has been a part of clinical care in the Philippines, there is little data on the EGFR mutation spectrum among Filipinos. This study aims to determine the frequency of EGFR mutations among a multiethnic Filipino population diagnosed with NSCLC in a tertiary care setting. **Methods:** A total of 626 tissue samples (444 biopsies, 108 pleural/ascitic fluids, 74 excision/resection), during a 15-month period (January 2015-March 2016) were assessed for the known EGFR driver mutations (exons 18, 19, 20, 21) using the Roche EGFR protocol with the Cobas Quantitative Real Time PCR. Macrodissection was performed as necessary. Patient demographics were recorded. Statistical analyses were performed using the Fisher's exact test. **Results:** Of the 626 samples, 52% were from males and 48% were from females. The median age was 64 years (range 13-94). The histologic classification were adenocarcinoma (92%), adenosquamous cell carcinoma (0.5%), NSCLC (3.1%), squamous cell carcinoma (SQCC) (4.1%), and adenocarcinoma with neuroendocrine features (0.3%). Tumor percentage ranges from less than 5% to 90%. Mutation was positive in 49.4%, negative (wild type) in 50% and invalid in 0.1%. Among the 307 EGFR mutated cases, 54.7% (n=168) had exon 19 deletion, 27.4% (n=84) exon 21 L858R, 7.2% (n=22) exon 20 insertion, 1.3% (n=4) exon

18 G719X, 0.7% (n=2) exon 18 L861Q, 0.3% (n=1) exon 20 T790M, and 8.4% (n=26) had dual mutations. Overall, presence of EGFR mutation was significantly higher in females (62% vs 38%) compared with males (two tailed p test, p<0.0001). Mutations in exon 19 deletion (p=0.0069) and exon 21 L858R (p<0.001) were significantly higher in females as compared with males. Among the 26 cases of SQCC, 9 cases showed EGFR mutation and 17 were wild type. Among the 9 EGFR mutated SQCC, 2 cases were positive for p63 (SQCC marker) and negative for TTF1 (adenocarcinoma marker) by immunohistochemical analysis. **Conclusions:** Here we report the largest EGFR mutation profiling data among Filipinos with NSCLC at a tertiary care setting, with a frequency of 49.4%. This prevalence is similar to those reported in Asia. EGFR is differentially mutated among NSCLC patients with different gender. Rare occurrence of EGFR mutation among cases diagnosed as SQCC histologically warrants inclusion of these cases for EGFR mutation screening. Further studies to correlate EGFR mutation patterns with other clinical characteristics, response to targeted therapy and overall survival in the Filipino population is warranted.

ST23. A Comprehensive DNA & RNA Pediatric Cancer Panel

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Introduction: We have created a comprehensive next generation sequencing based research gene panel for the potential precise diagnosis, prognosis, and potential therapy choice of childhood cancer that utilizes as little as 20 ng of DNA and RNA from FFPE, fresh, or frozen specimens. The panel detects gene mutations (44 full length genes, 82 hotspot genes), amplification (24 genes), InDels, expression (9 genes), and fusions (>1,400 variants). Results are available in as little as 3 days. Twenty-two gene targets as identified in the NCI MATCH program with matched therapeutic agents are included in the panel. In addition, the panel includes 49 of 51 features of interest requested by pediatric oncologists for clinical trials. **Methods:** We identified a comprehensive catalogue of genomic alterations reported in all common forms of pediatric cancer. In addition to personal experience and peer reviewed literature, the TF Compendia database was used to identify all known mutations (SNVs), InDels, gene amplifications, over-expressed genes, and gene fusions reported in pediatric cancer. Ion AmpliSeq™ primers covering all these features were designed for both extracted DNA and cDNA produced by reverse transcription of extracted RNA. Validation of individual analyte performance was confirmed by use of synthetic DNA targets and patient derived DNA/RNA from over 140 patient samples, including 47 leukemias, 6 lymphomas, 28 brain tumors, 4 neuroblastoma, and 33 sarcomas. Samples were chosen where orthogonal validation studies (FISH, CMAs, Sanger sequencing) had already confirmed a feature of interest. The analytic pipeline utilizes aligned BAM file output from the Ion Reporter software suite. A proprietary software suite, ICE, is used to identify and rank order by likely pathogenicity all variants, as well as to identify and quantitate sequence variants, InDels, gene amplification, gene expression, and gene fusions. **Results:** There was perfect concordance between previously identified gene fusions in all cases studied. An average of 2+ likely pathogenic variants were identified in each case. Over-expressed genes like MYCN in NB and MYC in Burkitt's lymphoma were readily identified. Gene amplification (n>7) was also identified in known positive cases. The most common defects were functionally related to chromatin configuration and transcriptional control; kinase mutations were uncommon. **Conclusions:** We have demonstrated the feasibility and utility of a broad pediatric cancer panel applicable to virtually any childhood malignancy. The assay identifies genetic alterations of all types in a broad spectrum of pediatric malignancies. The genetic landscape of pediatric cancer is distinct from that of adult cancer, with a low mutation rate and a preponderance of gene fusions.

ST24. Withdrawn

ST25. Specific and Reliable Detection of NTRK Rearrangements in IHC-Positive FFPE Samples of Solid Tumours by Nanostring Technology

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Introduction: Besides single nucleotide variants (SNVs), alterations such as *ROS1*-, *RET*-, *ALK*- and *NTRK*- fusions have been identified as oncogenic drivers in solid tumours. Those provide a great potential for therapeutic intervention. Hence, reliable and fast detection of rearrangements is of high interest and priority for patient care. However, the common fusions in the *ALK*-, *RET*- and *ROS-1* genes are currently identified by single-plex assays such as IHC or FISH. Therefore, alternative methods which allow multiplexed analysis of all relevant rearrangement targets need to be established. Especially the detection of *NTRK* fusions is of growing interest, since they are emerging as novel targets across various tumour entities. So far, no standard detection method has been implemented for *NTRK* fusions. Hence, novel technologies for the simultaneous detection of key target rearrangements in solid tumours should be tested. **Methods:** Nanostring´s nCounter Analysis is based on a digital colour-coded

barcode technology. The technology applies specific molecular probes to detect target regions within the gene of interest for various applications, such as 1) gene expression, 2) SNVs and 3) fusion events. RNA from formalin-fixed, paraffin-embedded (FFPE) solid tumour samples was isolated using the Promega16 LEV RNA extraction kit with an automated extraction (Maxwell, Promega). RNA quantity and quality was determined on a NanoDrop. Further, total RNA was hybridized to a multiplexed and custom-designed set of capture and reporter probes, targeting *ALK*, *RET*, *ROS1* and *NTRK1*,-*2*,-*3* rearrangements. Rearrangement status of tumour samples was determined beforehand with either IHC or FISH. **Results:** A customized multiplexed lung fusion detection assay could be validated, using solid tumour samples with known rearrangement status. Further, a prospective screening of unacquainted FFPE samples was conducted to identify fusions of *ALK, RET*, *ROS1* and *NTRK1, -2,-3* simultaneously. In addition, samples with discordant results for FISH and IHC were re-analyzed, using the customized nCounter Fusion Panel. A high concordance between IHC and nCounter-based detection of rearrangements was observed. **Conclusions:** The customized lung fusion assay represents a highly specific and reliable method for the simultaneous detection of *ALK*-, *RET*-, *ROS1*- and especially *NTRK1*, - *2*, -3 rearrangements in tumour-derived RNA from FFPE material. It provides an adequate platform for simultaneous detection of oncogenic fusions, thus representing a time-effective and reliable alternative to FISH.

ST26. Methylation Analysis of APC, AXIN2, DACT1, RASSF1A and MGMT Gene Promoters in Non-small Cell Lung Cancer

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Introduction: Non-small cell lung cancer (NSCLC) is still the leading cause of cancer-related mortality with poor prognosis due to diagnosis at advanced stage. Epigenetic alterations such as gene promoter hypermethylation play a key role in tumorigenesis and are considered as a possible marker for early diagnosis of lung cancer. Our aim is to determine promoter methylation in a panel of five tumor suppressor genes and their correlation with clinicopathological characteristics and EGFR /KRAS mutations. **Methods**: Sixty-six NSCLC cases were analyzed for gene promoter methylation of APC, AXIN2, DACT1, RASSF1A and MGMT genes using MS-HRM and pyrosequencing. EGFR/KRAS mutations were detected by pyrosequencing. Clinicopathological associations were assessed using SPSSv21 statistical package. **Results:** Overall promoter methylation was detected in 92% of the samples examined. APC promoter was the most frequently methylated promoter (63%), followed by RASSF1A (51.5%), MGMT (31%), DACT1 (29%) and AXIN2 (19%). 70% of the cases displayed methylation in one or more WNT pathway genes (APC, AXIN2, DACT1). Promoter methylation status of APC and DACT1 was correlated with histological type (p=0.015, p=0.017 respectively). Furthermore, 36.5% of the samples displayed methylation in one promoter, 31.7% in 2 promoters, 20.6% in 3 promoters and 3.2% in 4 promoters. None of the cases showed methylation of all 5 examined promoters. EGFR and KRAS mutations were detected in 10.2% and 12.5% of the cases respectively, only in adenocarcinoma subtype (p=0.006 and p=0.038). The presence of mutation was not associated with methylation status. AXIN2 gene promoter was not methylated in any EGFR/KRAS mutant samples. **Conclusions:** In our current study analyzing simultaneously methylation and EGFR/KRAS mutational status of NSCLC, gene promoter methylation emerged as an important mechanism of tumor suppressor inactivation in NSCLC. APC and DACT1 promoter methylation was related to adenocarcinoma histology, supporting the driving role of WNT pathway within this subtype. Methylation patterns and signatures will contribute to the understanding of NSCLC development and may provide a powerful tool for early diagnosis and therapy of lung cancer.

ST27. Expression Analysis of Wnt Pathway in Colorectal and Breast Cancer

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Introduction: Wnt signaling pathway plays essential roles during embryonic development and adult tissue homeostasis. Alterations in Wnt pathway components' expression can lead to dysregulation of the pathway and therefore to tumor development. Aberrant Wnt activation is frequently observed in human cancers, especially in colorectal cancer and breast cancer. **Methods:** Total RNA was isolated and reverse transcribed into cDNA from 75 fresh-frozen samples of colon cancer and 54 fresh-frozen samples of breast cancer as well as 10 normal adjacent colon and breast samples. The mRNA expression levels of WNT2, Frizzled 4 (FZD4), Frizzled 7 (FZD7), β-catenin (CTNNB1), TCF4 and LEF1 were determined by semi-quantitative Real Time PCR. Data were analyzed using the comparative ΔΔCt method. PPIA was used as reference gene. **Results:** Increased relative mRNA expression levels of WNT2, FZD4, FZD7, β-catenin, TCF4 and LEF1 genes were found in 60%, 29%, 38%, 16%, 33% and 32% of colorectal carcinomas, respectively. Interestingly, mRNA levels of TCF4 were associated with age (p=0,039). Statistically significant correlation among the expression of

several of the investigated genes was observed. As far as breast carcinomas are concerned, increased relative mRNA expression levels of β-catenin and LEF-1 were observed in 36% and 14% of the cases respectively, while decreased relative mRNA levels of WNT2, FZD4, FZD7 and TCF4 were found in 11%, 6%, 24% and 21%, respectively. Statistical significant correlations were established between increased mRNA of β-catenin and age (p=0,003), increased mRNA levels of LEF-1 and PR+ (p=0,027) and PR+/ER+ cases (p=0,025) and decreased mRNA levels of TCF-4 and HER-2+ samples (p=0,047). **Conclusions:** In conclusion, we observed dysregulation of Wnt pathway in colorectal and breast cancer. Interestingly, significant differences were found in the expression levels of the investigated Wnt pathway components between the two types of cancer. In colorectal cancer, statistically significant correlation of expression levels were observed among several Wnt pathway components, while in breast cancer statistically significant correlations with receptor positive cases were found.

ST28. High-throughput Sequencing-based Routine Clinical Diagnostics on Tumor Samples: Experience with the Illumina TruSight Tumor 15 Assay

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Introduction: High-throughput sequencing (HTS) is a powerful method for rapid and sensitive detection of relevant genomic variants in cancer, particularly when compared to time- and resource-consuming iterative single-gene testing methods. But there are many factors that need to be considered to ensure accurate and reproducible variant detection, as e.g. target region coverage, repetitive and/or low-complexity genomic sequences, long deletions and insertions (INDELs), variants exposed to the ends of reads, and trimming of primer sequences. Here, we report on the analysis of clinical samples processed with the amplicon-based Illumina TruSight Tumor 15 assay which is designed to provide a comprehensive assessment of 15 genes commonly mutated in solid tumors, based on formalin-fixed paraffin-embedded (FFPE) tumor samples. **Methods**: Three different diagnostic centres contributed 61 clinical tumor samples and 3 replicates of a publicly available control sample for which DNA libraries were prepared using the Illumina TruSight Tumor 15 kit, including intra- and inter-run replicates. Sequencing was carried out on Illumina MiSeq sequencers in 8 separate runs. We assessed the performance of this genetic test based on various metrics such as target region coverage, the detection of confirmed variants, and replicate reproducibility. **Results**: Target region coverage was very good, except for (1) samples that contained a significant amount of primer dimers, (2) a few amplicons that failed to provide good amplification in many or most samples, and (3) in rare cases where specific amplicons could not be amplified satisfactorily. The analytical pipelines detected nearly all the 202 Single Nucleotide Polymorphisms (SNPs) and INDELs that had been independently confirmed, with the only exceptions caused by low coverage in the respective samples. Reproducibility of variant calls in intra- and inter-run replicates was excellent with correlation coefficients of at least 98.8% between the variant fractions of replicate samples. Amplification (or copy number) analysis identified samples with elevated copy numbers for either EGFR, MET, or ERBB2 with apparent copy numbers of between 3.6- and 19.6-fold. **Conclusions**: The data and analyses presented here indicate excellent overall performance, good reproducibility, and informative amplification analysis achieved with the Illumina TruSight Tumor 15 kit for somatic tumor samples, as long as technical challenges such as primer dimer formation can be successfully controlled.

ST29. Clinical Evaluation of a Rapid Cell-free EGFR Mutation Detection Kit in Plasma from NSCLC Patients

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Introduction: The gatekeeper mutation T790M in the epidermal growth factor receptor (EGFR) is the most common cause of acquired resistance to EGFR kinase inhibitors, occurring in more than 50% of non-small cell lung cancer patients. Several third-generation EGFR kinase inhibitors that continue to be active in the presence of the T790M resistance mutation have shown promising results in early-stage trials. However, selection of candidates for these therapies requires invasive re-biopsy for tissue genotyping. The purpose of this study was to determine the per cent agreement between mutations detected in DNA isolated from tissue and plasma. Furthermore, a comparison of EGFR mutations detected prior to EGFR TKI therapy and post-progression were evaluated. **Methods:** Thirty-eight newly diagnosed patients with non-small cell lung adenocarcinoma were selected for this study. Ten milliliters of whole blood was collected from each patient at the time of diagnosis in cell-free DNA BCT tubes (Streck). Plasma was separated from the cellular fraction with double-centrifugation within 24 hours of collection. Circulating nucleic acids were isolated from plasma using the QIAamp Cirulating Nucleic Acid Isolation Kit (Qiagen). EGFR T790M, L858R and exon 19 deletion mutations were detected using the CTEGFR Mutation Detection Kit (EntroGen) on an ABI 7500 Fast real-time PCR instrument from approximately 5-20ng of cell-free DNA. Genomic DNA was isolated from formalin fixed paraffin embedded tissue from the same patients using the QIAamp FFPE DNA Isolation Kit (Qiagen) and EGFR

mutations were detected using the EGFR Mutation Detection Kit (EntroGen) on an ABI 7500 Fast real-time PCR instrument. Additionally, blood was collected from 8 patients who had progressed on EGFR TKI therapy and tested with the CTEGFR Mutation Detection Kit. **Results:** Twelve (32%) out of 38 tumor specimens were positive for EGFR mutations in tissue (8 exon 19 deletion, 4 L858R). Of the 12 tissue positive specimens, 6 were positive in plasma derived DNA. When stratified by tumor stage, the per cent agreement between tissue and plasma were 33% for stage I (2/6), 50% for stage II (2/4), and 100% for stage IV (2/2). Of the 8 patients who had progressed on EGFR TKI therapy, 7 (88%) still harbored the same EGFR sensitizing mutation detected at baseline (L858R or exon 19 deletion). Additionally, 3 out of the 7 (43%) had an acquired T790M resistance mutation. **Conclusions:** These preliminary results demonstrate the validity of using a plasma-based mutation detected test to identify patients who may respond to EGFR TKI therapy. Furthermore, the mutations detected at baseline (prior to therapy) are still present in plasma and tumors of patients at the time of progression.

ST30. IDH1/2 Mutations in Gliomas: A Single Tertiary Cancer Institutional Experience

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Introduction: Isocitrate dehydrogenase (*IDH*) mutation is characteristic of diffuse gliomas (DG) and secondary glioblastomas (GBM). **Methods**: FFPE cases of Glioma analyzed for *IDH1/2 mutation* by Sanger sequencing (n=687). Some were additionally evaluated for ATRX protein, *1p19q* deletion and *EGFR* amplification. All cases were reclassified based on 2016 WHO classification. Oligodendroglial (ODG) tumors with incomplete molecular evaluation (for *1p19q* deletion/ATRX) were classified as ODG-NOS. Cases with discordance between histology and molecular findings were classified as glioma, NOS-astrocytic (GNOS-A) and ODG phenotype (GNOS-ODG) respectively. **Results**: Of these 665 were interpretable for *IDH1/2* sequencing; 22 pediatric [<6yrs:2, 6-14yrs:11, 15-18yrs: 9] and 643 adults. Histological spectrum in adults - 632 DG {astrocytomas (DA:20), anaplastic (AA:78), ODGs [(ODG:36, ODG-NOS:6), anaplastic ODG (ODG-A:116, ODGA-NOS:21)], GBM:213, high grade glioma-NOS [HGGNOS-A:50, HGGNOS-ODG:58], low grade glioma-NOS (LGGNOS-A:14 & LGGNOS-ODG:17)}, other astrocytomas [pilocytic astrocytoma (PCA:6), PXA: 2] and glioneuronal tumours [ganglioglioma: 2, extraventricular neurocytoma: 3, CD/DNET:1]. Pediatric group comprised of 17 DGs [AA:2, ODGA-NOS:1, GBM:7, HGGNOS-A:2, HGGNOS-ODG:1, LGGNOS-A:3 & LGGNOS-ODG:1], 4 PCA and 1 CD/DNET. *IDH* mutation was seen only in DGs. In pediatric, 3 cases (1/1 ODG-A & 2/7 GBMs) of age group 15-18yrs were mutated and in adult 61.7% [19-25yrs (40.5%; 15/37), 26-40yrs (73.3%; 176/240), 40-60yrs (62.0%; 171/276), >60yrs (35.4%; 28/79)] were mutated. The most frequent mutation was *IDH1R132H* (78.1%) followed by IDH1R132C (5.3%), IDH1R132S (4.3%), *IDH1R132G* (3.3%)
and *IDH1R132L* (1.3%*)* with *IDH2* mutations in small percentage of cases [6.4% *IDH2R172K, 0.8% IDH2R172M*, 0.3% *IDH2R172G,* 0.3% *IDH2R172W*]. In adult group, *mIDH* was seen in 70% (14/20), 83.3% (65/78), 100% (36/36), 100% (6/6), 100% (116/116), 100% (21/21), 24.0% (51/213), 42% (21/50), 74.1% (43/58), 57.1% (8/14) and 53.0% (9/17) cases of DA, AA, ODG, ODG-NOS, ODG-A, ODGA-NOS, GBM, HGGNOS-A, HGGNOS-ODG, LGGNOS-A and LGGNOS-ODG respectively. One case of AA showed somatic homozygous *IDH1R132H* mutation. *1p19q* was evaluated in 36/42 ODGs, 116/137 ODG-A cases, 8/17 LGGNOS-ODG and 47/58 HGGNOS-ODG. The former two groups only showed co-deletion. *EGFR* FISH was performed on 90/220 GBMs with 24 amplified (all *wtIDH*) and 66 non-amplified (*mIDH*:14, *wtIDH*:52). **Conclusions:** *IDH1/2* mutation was noted in 61.7% of adult DGs and can also be detected in older pediatric DGs. 9.1% of adult histological pure ODG showed *wtIDH1/2*. *IDH* mutations are heterozygous but in this study one case showed homozygous mutation, which is exceptionally rare, the implications of which needs to be further studied.

ST31. IQFISH Lung Cancer Panel Probes on DAKO Omnis - A Walkaway Automation for Lung Cancer FISH

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Introduction: Lung cancer is the leading cause of cancer related deaths. Non-small cell lung carcinoma (NSCLC) is a type of epithelial lung cancer that accounts for approximately 85% of all lung cancers. Recurrent chromosomal alterations involving *ALK, ROS1, RET* and *MET* have been identified as important drivers of tumor growth in NSCLC. Fluorescence in situ hybridization (FISH) is a powerful method for visualizing such changes in genome architecture and therefore serves as an important molecular diagnostic tool in NSCLC. Performing FISH on formalin fixed paraffin embedded (FFPE) samples from NSCLC patients, however, is laborious and requires extensive hands-on time (two days or more). To reduce this burden, we have developed a fully automated FISH workflow where multiple samples can be processed at any given time for the detection of *ALK, ROS1*, and *RET* rearrangements and *MET* amplification in FFPE lung cancer specimens. FISH processing utilizing the Dako Omnis staining platform in conjunction with the novel, non-toxic (formamide- free), IQFISH rapid hybridization buffer reduces total processing time to as little as 4 hours. **Methods**: FISH probes were designed to avoid repetitive

sequences and maximize coverage of gene-specific regions. Probe synthesis was performed using Agilent's novel Oligonucleotide Library Synthesis (OLS) technology. Staining performance on DAKO Omnis was evaluated across eight FFPE lung cancer and two needle biopsy samples. Probe signal intensity and signal patterns were evaluated and compared to manually stained slides. Statistical analysis was used to compare the performance of the manual and automated staining. **Results:** FISH analysis of gene rearrangements in *ALK, ROS1* and *RET* and gene amplification in *MET* was successful for all of the samples processed on the Dako Omnis. The probe staining signal intensity was comparable to the manually processed samples (Student's t-test, $p>0.05$). The staining performance was consistent across multiple runs and Dako Omnis instruments (Max %CV: 15%). For all probes, the observed signal patterns were concordant between Dako Omnis and manually stained samples on both normal and abnormal samples. Further, the performance of all the probes was robust when evaluated across multiple slide types and section thicknesses using commonly available microscope/filter combinations. **Conclusions:** Combining the Dako Omnis platform with IQFISH hybridization buffer and Agilent's probe technology provides rapid, robust and reproducible FISH staining while removing the many workflow bottlenecks associated with traditional processing of tissue samples.

ST32. Absence of MGMT Promoter Hypermethylation in H3 K27M-mutated Diffuse Midline Gliomas WHO Grade IV

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Introduction: Glioblastomas WHO grade IV (GBM) are malignant astrocytomas with necrosis and/or vascular proliferation that mostly have a supratentorial and much rarer an infratentorial location in the brain stem or in the myelon. The revised fourth edition of the WHO classification of brain tumors from 2016 is defining such infratentorial astrocytomas as diffuse midline gliomas WHO grade IV H3 K27M-mutated (DMG) if they carry a mutation in the histone variant encoding genes H3F3A or HIST1H3B position K27. The current standard of care for GBM patients is total surgical resection followed by combined radio- and chemotherapy with temozolamide (TMZ). However, often surgical resection of brain stem GBM is strongly limited due to vulnerability of the anatomical structures so patients only receive radio- and chemotherapy. Hypermethylation of the promoter of the MGMT gene is an established predictive marker for TMZ treatment. Around 40% of patients with a supratentorial GBM carry MGMT promoter hypermethylation and respond to TMZ treatment. **Methods:** A series of 129 patients with an astrocytoma of the midline was retrieved from the archive. All cases were reexamined histologically and tumor areas were marked for manual microdissection if necessary. After DNA extraction codon 27 and 34 of the H3F3A gene and codon 27 of the HIST1H3B gene were analyzed by pyrosequencing. For MGMT promoter hypermethylation DNA was bisulfite treated and 11 CpG sites in the MGMTpromoter were analyzed quantitatively using pyrosequencing, using 10% methylation level as threshold for scoring a sample as "hypermethylation". **Results:** All specimens could be analyzed successfully. Thirty-eight specimens (29%) were positive for a H3F3A mutation. All H3F3A mutated cases in this series displayed the K27M mutation, H3F3A codon 34 mutations were not found. None of the analyzed astrocytoma of the midline displayed MGMT promoter hypermethylation. **Conclusions:** These results indicate that i.) At least a biopsy should become established to determine the genetic status of a midline glioma, ii.) MGMT promoter hypermethylation is a rather rare event in DMG and iii.) Based on the concept that MGMT promoter hypermethylation is associated with strongly reduced expression of the O-6-methylguanine-DNA methyltransferase protein, DMG patients presumably have no benefit from TMZ treatment and are only exposed to cytotoxic side effects.

ST33. Detection of Somatic Alterations in Circulating Cell-free DNA of Esophageal Carcinoma Patients during Primary Staging is Predictive for Post-surgical Tumor Recurrence

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Introduction: Esophageal cancer (EC) is among the ten most common malignancies worldwide. Especially esophageal adenocarcinoma spreads rapidly throughout the western countries. Genomic profiling of primary tumor foci and detection of somatic mutations in circulating cell-free DNA (ccfDNA) of patients with EC during the course of treatment will probably play a major role in the future. The present study is therefore the first to determine the feasibility of using liquid biopsies for genomic profiling in EC patients, to compare two different technologies for detection of mutations in ccfDNA and to evaluate the clinical impact of detectable somatic alterations in ccfDNA during primary staging in EC patients. **Methods:** In 20 patients with locally advanced EC routine endoscopic tumor biopsies and simultaneous blood samples were taken at the time of primary staging. Biopsies were histologically characterized by a senior pathologist and cryo-preserved. Plasma was gained from whole blood

samples immediately after sampling and stored at -80°C. Genomic DNA from frozen tumor tissue as well as cell-free DNA from plasma samples were extracted and analyzed for mutational status of 12 genes using an Ion AmpliSeq Custom Next-Generation Sequencing DNA Panel (ThermoFisher Scientific) on the MiSeq system (Illumina). Further, digital droplet PCR (ddPCR) using the QX200 Digital Droplet System (Bio-Rad) was performed on both, tissue and plasma samples. Genetic data was then correlated with the patients' outcome with a median follow-up of 13.9 months. **Results:** In 76% of the analyzed EC tumor biopsies at least one somatic mutation was detected with next-generation sequencing (NGS) using the 12 gene panel. All mutations detected by NGS were also detectable by ddPCR with a similar allele frequency. In contrast, mutations in ccfDNA were detected only in the minority of patients using NGS (12%). ddPCR was capable of detecting mutations in ccfDNA in two additional patients indicating a significant higher sensitivity for detection of somatic alterations in ccfDNA of EC patients. Two patients developed tumor recurrence resulting in tumor related death in one patient. Remarkably, both patients with recurrent tumor had detectable somatic mutations in ccfDNA during primary staging. **Conclusions:** Liquid biopsies represent a non-invasive method for the identification of somatic mutations. This is the first study to show a higher sensitivity for ddPCR compared to NGS in detecting mutated ccfDNA in EC patients. Detection of somatically altered ccfDNA in EC patients during primary staging is of major clinical relevance as it seems to be predictive for tumor recurrence after surgery.

ST34. Genomic Data Illustrates Informative Roles for Gene Expression and Methylation Profiles for Tumor Classification

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Cancer classification in the clinic is primarily based on histo-morphologic analysis in the proper clinical context, supplemented by immunohistochemical and occasional molecular studies. Recent genomic studies of cancer show the utility of integrated multi-omics platforms for molecular classification. An unexplored question relates to the relative utility and accuracy of individual molecular platforms to aid in tumor classification. To address this, we investigated molecular platforms and signatures from TCGA data (n=6216 tumor samples) and showed that mRNA and DNA methylation data each show good accuracy, as single platforms, for tumor classification, using similarity network fusion analysis. We find that when combined, mRNA and methylation together show a modest improvement in classification accuracy and revealed a set of cases for which the mRNA- and methylation-based molecular signatures suggested a classification that differed from the original histologic diagnosis, in a manner that was concordant. A number of these outliers remained consistent after using alternative classification algorithms (Random Forest, hierarchical clustering), as well as from spectral clustering with an alternative molecular platform (microRNA). Mutational profiles of a subset of informative cases provided independent evidence consistent with the methylation and mRNA signatures, providing consistent molecular evidence for outlier cases. Overall, the results indicate that unsupervised approaches with individual genomic platforms, especially gene expression and DNA methylation, provide substantially useful classification information for the determination of tumor types and molecular subtypes, identify occasional outlier cases in which the molecular signature is distinct from signatures expected for a given histologic diagnosis and provide a framework for the development of genomic tools to aid in tumor classification.

ST35. Comparison of Two Testing Methodologies for EGFR Mutation Screening of cfDNA from Lung Cancer Patients

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Introduction: About 50% of patients with advanced non-small cell lung cancer (NSCLC) with an acquired resistance to epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKI) display an EGFR T790M mutation. The introduction of third-generation EGFR-TKIs targeting T790M mutated tumors has generated a need for plasma genotyping of *EGFR*. In this study, we compared real-time PCR and next generation sequencing (NGS) as diagnostic tools to detect *EGFR* mutations in circulating cell free DNA (cfDNA). **Methods:** CfDNA was extracted from plasma originating from lung cancer patients with sensitizing *EGFR* mutations (n=26) and analyzed for *EGFR* mutations using real-time PCR (*therascreen*® EGFR Plasma RGQ, Qiagen) and NGS (Accel-Amplicon™ EGFR Pathway Panel from Swift Biosciences, Illumina MiSeq). The two methods were also evaluated using reference standard DNA with known mutation frequencies. **Results:** The extracted cfDNA displayed a satisfactory quality and quantity to generate genotyping data from both methods. Mutations in *EGFR* could be detected in 13 of the 26 samples demonstrating that circulating tumor DNA was present in the extracted cfDNA. The two methods generated concordant genotypes for 23 of the 26 samples. For two samples a deletion in exon 19 of *EGFR* was identified using *therascreen*® EGFR Plasma RGQ. In these two samples, the NGS sequencing data also indicated an exon 19 deletion but with a low variant allele fraction (0.2 and

0.4%). Furthermore, one of the analyzed samples displayed an exon 19 deletion and a T790M mutation in *EGFR* when analyzed with NGS but these mutations did not pass the cutoffs for true positive calls when the sample was analyzed using the *therascreen*® EGFR Plasma RGQ kit. **Conclusions:** With the QIAamp Circulating Nucleic Acid Kit it was possible to extract cfDNA of satisfactory quality and quantity for subsequent *EGFR* mutation analysis for all 26 patient samples in the study. Both investigated methods were found suitable for detection of *EGFR* mutations in cfDNA from lung cancer patients.

ST36. A Patient-Ike Circulating Tumor DNA (ctDNA) Reference Material

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Introduction: We developed and evaluated a novel ctDNA reference material that was designed to mimic certain key characteristics of native cell-free DNA (cfDNA) purified from plasma. Commutability is critical for a reference material, and many existing methods of preparing ctDNA analogues result in material that is not well-matched to clinical specimens. For example, sonication of genomic DNA yields fragments across a relatively broad size distribution, and results in material that must be used at significantly higher input than native ctDNA; mechanical shearing damages DNA and can reduce the efficiency of adapter ligation, even after enzymatic end-repair. The result is poor data quality on assays for which target enrichment depends on efficient adapter ligation, such as many hybrid/capture-based Next Generation Sequencing (NGS) assays. Higher DNA input amounts must be used for these sonicated materials to achieve the same sensitivity as for native ctDNA. **Methods:** We created a commutable ctDNA reference material by starting with synthetic variant-containing DNA sequences and mixing at defined ratios with genomic DNA from GM24385 cells. The use of GM24385 as the wildtype background material allows for the comparison of detected variants against the NIST HG002 high confidence variants. The use of synthetic DNA enables a high degree of multiplexing for large numbers of cancer-associated variants at defined frequencies. Fragmentation was followed by size-selection in order to obtain ctDNA-like sizes. Additional steps were used in order to increase the yield of output material from the size-selection step. The general method of preparing the reference material is also compatible with native cfDNA, such as that isolated from cancer patients. **Results:** The reference material was submitted to several testing laboratories along with similar material that had been prepared by sonication alone. When tested using hybrid/capture-based assays at similar DNA input amounts, variant detection was superior in the novel reference material compared to sonicated DNA. **Conclusions:** Therefore, we conclude that our new ctDNA reference material has greatly improved commutability compared to materials that consist of sonicated DNA.

ST37. Experience of Cancer Genomic Profiling of Hong Kong Chinese Cohorts

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Introduction: The incidence rate of cancer is around 30,000 annually in Hong Kong. Comprehensive genomic profiling is one of the possible methods in substituting the debatable difference in cancer management. With the extra genomic information, a more diversified treatment approach can be adopted. This study is aimed at analysing the extra genomic information and advocate innovative cancer management. **Methods:** Two hundred and four samples were taken for comprehensive genomic profiling. A gene panel of 315 oncology genes were analysed in the samples from patients with different disease types. The genetic profiles were then analysed based on disease types and different kinds of genetic mutations. **Results:** Among 204 samples, there were 65 samples from lung cancer, 16 from colorectal cancer and 11 from breast cancer. Others were from other gastrointestinal carcinomas, soft tissue sarcoma and other less common cancers. EGFR alternations are found in 47.7% of lung cancer patients. Other alternations, which are potential actionable, were also found. They included PIK3CA (16.9%), MET (9.2%), ALK (9.2%), ERBB 2 (9.2%), ROS1 (3.3%), RET (1.5%) and ERBB3 (1.5%). Some commonly found genetic mutations were found in colorectal cancer patients and the prevalence rate are as follow, KRAS (50%), BRAF (12.5%) and EGFR (6.3%). In the breast cancer samples, a wider range of genetic mutations were also found. PIK3CA (15.4%), ERBB2 (15.4%), FBXW7 (7.7%), AKT3 (7.7%), AKT1 (7.7%), PTEN (7.7%), AR (7.7%) and EGFR (7.7%) were found in the breast cancer cases. **Conclusions:** In the routine cancer biomarkers screening, particular biomarkers are tested for its specific cancer type(s), like ERBB2 overexpression in breast cancer. However, the use of off-label target drugs may serve the ground of drug choices. Another major finding of our study is the multiple actionable genetic mutations governing different proliferation pathways in the same patients. Attempt of the combination target drugs may potentially increase the response rate of the treatment, longer duration of response or even prolonged survival. Our studies are based on samples in Hong Kong Chinese population, the result found in our studies may not be the same as other countries. It can help the understanding of cancer genomics in Hong Kong Chinese population as well as contrasting the difference in different ethnic groups. Comprehensive genomic profiling can then offer insights to patients beyond disease type and

ethnic group and bring cancer treatment to the next level. Our study demonstrates the use of comprehensive genomic profiling may become a prerequisite for precision cancer patient management.

ST38LB. Sensitive Sequencing Method for Detection of Actionable Cancer-driver Somatic Mutations in Specimens with Scarce Material

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Introduction: Somatic mutations emerge as superior biomarkers for the prediction of efficacy of targeted drugs, and are key to personalized medicine, *i.e.* rationalized match of anti-cancer drugs to cancer patients. For 15-30% of cases, the amount of starting tissue and DNA material is a limiting factor with current methods, typically requiring 50ng gDNA. In some of the cases, all material left after routine pathological assessment (mostly immunohistochemistry, IHC) are stained slides. We developed a series of methods that would allow detection of critical amino-acid substitutions (EGFR L858R, EGFR T790M, BRAF V600E, KRAS G12, etc.), gene fusions (BCR-ABL, etc.) and copy number variations (MYC, CCNE1, etc.), from scarce and pre-stained material. **Methods**: We assessed the sensitivity and specificity over a common genomic area, encompassing all frequently mutated exons of over 150 cancer causing genes, using a unique combination of target enrichment tools on Illumina® HiSeq2500. The main sensitivity derives from an ultra-sensitive library construction method, prior to the target capture. This combination improves the sensitivity to less than 1 ng gDNA, thus enabling cancer somatic mutation detection from micro-dissected malignant cells from FFPE sections, based on H&E stained slides. Furthermore, even if the starting material was pre-stained, we could detect the mutations. In addition, we combined the target somatic mutation detection with RNA detection (NanoString) that allows identification of key gene fusions, and instructive gene expression signatures, related to the efficacy prediction of immunological checkpoint inhibitors. **Results**: In our hands, a trend was observed, in which increased starting material improves detection of somatic mutations and representation of sequences. Nonetheless, the technology we describe does detect sensitivity, reproducibility and specificity, largely comparable to standard target enrichment commercial kits. **Conclusions:** These results suggest that with cases where the source material is limiting or extensively corrupted by previous analyses (IHC), clinically driven tumor sequencing should employ the more sensitive library construction kit before the target capture step.

ST39LB. Identification of Biomarkers for Prostate Cancer by DNA Methylation Analysis

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Introduction: Prostate cancer is the second leading cause of cancer related mortality in men. Elevated levels of prostate specific antigen (PSA) are detected in serum of prostate cancer patients, which is currently used to screen for the presence of prostate cancer in elder men. However, the use of PSA as biomarker for prostate cancer has several limitations. PSA levels increase not only in patients with prostate cancer but also under certain benign conditions, resulting in a high level of false positives. This and other limitations make novel biomarkers with high specificity for prostate cancer a necessary target for research. DNA-hypermethylation of CpG-islands in the promoters of tumor suppressor genes frequently occurs in tumors, resulting in epigenetic silencing. Changes in DNA-methylation can be measured, making hypermethylated genes promising new biomarkers for prostate cancer detection. **Methods:** The genome-wide DNA-methylation profiles of primary prostate cancer and adjacent normal tissue from patients was assessed by using the Infinium HumanMethylation450 BeadChip from Illumina®. Methylation-specific qPCR (qMSP) was used to validate the hypermethylation of the top significant differentially methylated genes in an independent patient cohort. To investigate the biological function of one of the target genes, the CRISPR-Cas9 method was used for the generation of knockout cell lines as well as stable transfection of prostate cancer cell lines to investigate overexpression of the respective gene. Invasio assays were performed using BD BioCoat™ Matrigel™ Invasion chambers. **Results:** The DNA-methylation profiles of primary prostate cancers were compared to normal adjacent tissue. Principal component analysis of these methylation data clustered the samples into two distinct groups, based on the differentially methylated CpG sites. We identified several promoter regions of genes that were hypermethylated in prostate cancers as compared to normal adjacent tissue. These were successfully validated in an independent cohort with a bigger sample size using qMSP. Among the top significant differentially methylated genes was *SERPINB1* which was found to have a possible role for prostate cancer progression. We identified a negative correlation between *SERPINB1* expression and the invasive potential of different prostate cancer cell lines. **Conclusions:** Differential methylation between prostate cancer and normal prostate epithelium allows for the clear classification of prostate cancer and normal tissue and provides insight into biologically relevant targets for

prostate cancer development. As a next step we aim to design assays for non-invasive detection of our epigenetic biomarker panel in liquid biopsies and urine samples of prostate cancer patients.

ST40LB. Mutational Analysis of FOXL2 Gene Assisting in the Diagnosis of Recurrent Adult Granulosa Cell Tumor with Unusual Morphology

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Introduction: The diagnosis of ovarian adult granulosa cell tumor (AGCT) is based on morphology with ancillary immunohistochemistry (IHC) when necessary. Recurrent AGCT usually shares morphologic features with the primary. Most AGCTs harbor mutation in the forkhead box L2 (*FOXL2*) gene (c.402C>Gp.C134W) which is unique for this tumor. Herein we present a case of recurrent AGCT with unusual morphology and IHC profile and report molecular findings that enabled diagnosis of recurrence. **Methods:** H&E stained slides and IHC preparations were reviewed**.** NGS-based analysis of FFPE tumor was performed using the Oncomine cancer panel (ThermoFisher Scientific). Peripheral blood DNA was used as a germline control. Clinical information was extracted from the medical records. **Results:** The patient was diagnosed with AGCT, stage 1C at the age of 37 years. Ten years later, the tumor recurred in the pelvis displaying morphologic features identical to those of primary. The patient underwent two debulking surgeries over a course of one year with histologically confirmed AGCT recurrence and was treated with chemotherapy, followed by maintenance hormonal therapy with stable findings on imaging studies for six years. Upon evidence of progression on CT imaging, the patient was treated with chemotherapy. After initial interval improvement, progression was detected. Exploratory laparotomy revealed tumor involving intraabdominal sites. Optimal cytoreduction was achieved. Pathologic examination revealed a malignant neoplasm with spindled and epithelioid features and high grade nuclei. No morphologic similarities to the primary tumor were noted. IHC work up (tumor cells positive for vimentin, EMA, CK7, MOC31, very focally positive for calretinin, thrombomodulin, CD56; BAF-47 (INI) retained; negative for inhibin, desmin, SMMS, BerEP4, ER, PR, GATA-3, PAX8, CK5/6, DOG-1, SMA, myoD1, myogenin, S-100, CD31, CD34, ERG, CDX2, GCDFP-15, TTF-1, SOX-10, CD99, HMB-45, S-100) did not allow for a definitive classification of the tumor. Mutational analysis demonstrated somatic mutations in *FOXL2* (NM_023067.3 (FOXL2):c.402C>Gp.C134W); *NRAS* (NM_002524.4(NRAS):c.34G>Ap.G12S); *PIK3CA* (NM_006218.2(PIK3CA)c.1624G>Ap.E542K. Clinical course was complicated by ascites and small bowel obstruction. The patient died of progressive disease 18 years after initial diagnosis, with recurrent disease present for the last 8 years. **Conclusions:** Mutational analysis of *FOXL2* can assist in the diagnosis of AGCT when morphologic features are ambiguous. Presence of *FOXL2* c.402C>Gp.C134W somatic mutation confirms that the metastatic tumor is related to the ovarian AGCT and represents its recurrence. NGS-based sequencing may allow for discovery of targetable alterations that could be relevant in selecting therapy.

ST41LB. Molecular Profiling of Epidermal Growth Factor Receptor in Tunisian Patients with Non-small Cell Lung Cancer Using Real Time PCR

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Introduction: Despite recent advances, non-small cell lung cancer remains a devastating disease and carries a grim prognosis. For appropriate treatment selection, the updated Guidelines recommend broad molecular profiling for all patients with adenocarcinoma disease. As a consequence and in contrast with 10 years ago, precise histological subtyping and targeted Epidermal Growth Factor Receptor (EGFR) testing is now mandatory. **Methods:** Herein, we assessed the EGFR mutation status of 19 formalin fixed-paraffin embedded samples of lung adenocarcinomas. Mutational analysis concerned exons 18 to 21 of EGFR by Real-time polymerase chain reaction (RT-PCR) using the Therascreen EGFR RGQ PCR mutation kit. **Results:** Activating EGFR mutations were found in 2 men among 19 patients (10.5%). In one case the mutation was an exon 20 insertion detected in a lepidic adenocarcinoma. In the other it was a G719X (A/S/C) point mutations in exon 18, found in a solid adenocarcinoma. **Conclusions:** Frequency of EGFR mutations in pulmonary adenocarcinomas of our series is similar to that found in the European ones with some particularities. The mutations detected are uncommon and were associated with male gender. Further studies with larger Tunisian series are required to obtain more conclusive results.

ST42LB. External Quality Assessment for Molecular Pathology: Lessons Learned and the Need for Continued Quality Improvement

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Introduction: The promise of personalised medicine for all has been a major driver in the rapid evolution of the field of molecular pathology. This need has been met by labs from different diagnostic environments, predominantly in the fields of pathology and genetics. Labs have been working to set up testing for tumour markers and manufacturers have responded by developing new diagnostic kits/end-to-end diagnostic solutions to meet this need. However, accuracy of genotyping and interpretation is essential for the realization of the promise of personalized medicine. External Quality Assessment (EQA) schemes are needed to ensure that the quality of testing delivers the right result, for the right patient, in the right time. **Methods:** The European Molecular Genetics Quality Network (EMQN) is an organization promoting quality in molecular testing worldwide by providing EQA schemes. These include schemes for molecular pathology, monogenic disorders and technical approaches to testing. The objective is to establish inter-laboratory comparability. The process establishes and standardises best practice in correctly identifying sequence variants, interpretation of the results and clerical accuracy. EMQN sent identical clinically relevant samples to each EQA participant which they tested using their routine methods. The anonymised results were peer reviewed and made available to all participants in order to enable comparisons between laboratories and assess individual laboratory performance. **Results:** The 2016 schemes recently concluded and our presentation will summarise the data from 6 years of EQA showing the rate of serious diagnostic error remains stubbornly high (mean of 3.65%). Errors are made by labs using a broad range of methodologies. Analysis of error patterns indicates that poor validation of new tests contributes significantly, especially when implementing an NGS strategy, or using a "black box" commercial diagnostic solutions. Over 6 years, there has been significant improvement in the clinical reporting of results with far less over interpretation of the genotyping results and improved practice with respect to the nomenclature used for the reporting of sequence variants. Improvement in the quality of testing is evident but there remains more to do. **Conclusions:** We conclude that annual participation in EQA can improve the quality of testing in molecular pathology and contribute to the achievement of the promise of personalised medicine.

ST43LB. Identification of New Molecular Pathways Involved in Endometrial Cancer Progression

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Introduction: Endometrial carcinoma is the most common malignancy of the female genital tract. The characterization of a very large cohort of human endometrial cancer tissues showed that, although PI3K-mTOR pathway activation and p53 inactivation play different roles in the initiation of different cancer subtypes, co-occurring alterations in both signaling pathways represent a frequent unifying pathogenic feature of late stage tumors of all subtypes. We hypothesize that, these co-occurring events result in a condition of oncogene addiction leading to cancer progression and poorer prognosis. We aim at identifying and characterizing proteins crucial for the survival of cancer cells with this molecular signature (PI3K-mTOR pathway activation and p53 inactivation), as they may represent new prognostic and potentially predictive markers for personalized medicine. **Methods**: We designed a PI3K pathway-dependent synthetic lethality screen based on RNAi technology and used TAL-effector nuclease genome editing tool to knock-out *PTEN* (an important inhibitor of the PI3K pathway) in *TP53*$^{-/-}$ endometrial carcinoma cell lines. To confirm the aberrations critical for disease progression we planned to investigate the obtained results using the samples of the human endometrial cancer cohort. Thus, we designed a targeted re-sequencing experiment with Ion Torrent platform and IHC characterization of the corresponding tissue microarrays. **Results**: Screening of target genes for *PTEN* synthetic lethality led to the identification of several candidate genes. In parallel, we investigated the mutational status of screening hits in the human endometrial cancer cohort and analyzed the results considering the immunohistochemical characterization available (microsatellite instability status surrogate markers, p53 and PI3K-mTOR pathway activation status). **Conclusions:** In order to identify new biomarkers for precision medicine, the molecular mechanisms leading to synthetic lethality will be further investigated in view of the different genetic alterations identified in the human samples.

ST44LB. Next Generation Sequencing of Malignant Mullerian Mixed Tumours Identifies Gene Alterations with Potential Clinical Actionability

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Introduction: Malignant Mixed Mullerian Tumours (MMMT) are aggressive neoplasms which are histologically composed of carcinoma and sarcoma elements. Although rare, they account for 16.4% of all deaths caused by uterine malignancies and have a 5-year survival rate of 25-30%. Also, they are characterized by a poor response to common radio- and chemotherapy regimens. The high rates of recurrence and metastases require the identification of new and more effective therapeutic options. **Methods:** In this study, a tissue microarray (TMA)

of 21 MMMT cases was constructed. The sarcomatous and carcinomatous components were punched separately and subsequently characterized by immunohistochemistry to investigate a large number of tumor antigens. Targeted next generation sequencing of the carcinoma and sarcoma components was performed using the Oncomine™ Focus Assay with Ion Torrent™ sequencing platform (Thermo Fisher Scientific). This commercially available assay allows to analyze hotspots, SNVs, Indels, CNVs, and gene fusions targeted by oncology drugs and published evidence. The variants detected were further analyzed with the Oncomine™ Knowledgebase Reporter (Thermo Fisher Scientific), a bioinformatics tool that contains curated information about published therapeutic options and ongoing global clinical trials. **Results:** The immunohistochemical staining of the TMA allowed us to perform a comprehensive characterization of the selected cases with emphasis on markers of epithelial to mesenchymal transition. As for oncogenes and possible targetable alterations, overexpression of p53 in 38% and microsatellite instability in 19% of cases were of particular interest. The most frequently altered genes detected by Oncomine™ Focus Assay were *KRAS* (44% of samples), *PI3KCA* (37%), *HRAS* (7%), *MAP2K2* (7%), and *ERBB3* (7%). Private high impact variants occurring in *other genes* have also been found. Most of the genetic alterations detected were clonal in nature. **Conclusions**: The comparison of the molecular profiles in the carcinoma and sarcoma components is consistent with the theory of a clonal origin and supports the hypothesis that MMMTs represent a sarcomatous transformation/transdifferentiation of endometrial carcinoma. Most of the detected gene alterations represent specific actionable targets which may improve future clinical management of these neoplasms.

ST45LB. ALK-IHC is a Better Predictor for Outcome After ALK Inhibition with Crizotinib than ALK-FISH in Stage IV Non-small Cell Lung Cancer

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Introduction: ALK rearrangement detection using fluorescence in situ hybridization (FISH) is routinely applied to identify non-small cell lung carcinoma (NSCLC) patients eligible for treatment with ALK inhibitors. Recently ALK immunostaining in resectable NSCLC showed predictive value. We evaluated tumor response rate and survival after crizotinib treatment of advanced NSCLC patients with ALK activation using both immunohistochemistry (IHC) and FISH. **Methods:** Stage IV NSCLC patients treated with crizotinib were selected and tumor response was assessed. ALK rearrangements were assessed by FISH (Vysis ALK-Break-Apart FISH-Probe KIT) and dichotomous IHC (Ventana ALK-D5F3-CDx assay: positive vs negative). Subsequently, validation was carried out using cohorts of ALK-FISH-positive stage IV NSCLC patients from 4 other hospitals. **Results:** Twenty-nine consecutive patients, showing ALK-positive advanced NSCLC determined by FISH and/or IHC on small biopsies or fine needle aspirations (FNA), were treated with ALK inhibitors. All ALK-IHC-positive patients responded to crizotinib except for three with primary resistance. No tumor response was observed in 13 ALK-FISH-positive, ALK-IHC-negative patients. This finding could be confirmed in an external cohort of 16 patients. ROC curves for ALK-IHC and ALK-FISH compared to treatment outcome, showed that dichotomous ALK-IHC outperforms ALK-FISH (tumor response AUC 0.86 vs. 0.64, p=0.03; PFS AUC 0.86 vs. 0.36, p=0.005; OS AUC 0.78 vs. 0.41, p=0.01, respectively). **Conclusions:** Dichotomous ALK-IHC is superior to ALK-FISH on small biopsies and FNA to predict tumor response and survival to crizotinib for stage IV NSCLC patients. Our data indicate that dichotomous ALK IHC should be considered as standard companion diagnostic test to select NSCLC patients that benefit from ALK-targeting therapy.

ST46LB. MET Overexpression, Amplification and Exon-14 Skipping in Patients with Resected NSCLC: Prevalence and Clinical Association Results from the European Thoracic Oncology Platform Lungscape Project

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Introduction: In a well-defined non-small cell lung cancer (NSCLC) cohort of the European Thoracic Oncology Platform (ETOP) Lungscape program, we examined the epidemiology of MET protein overexpression and gene amplification, their inter-correlation, and their association to outcome. **Methods:** Surgically resected NSCLC were analyzed for MET gene copy number (GCN) and expression using silver in-situ hybridization (SISH) and immunohistochemistry (IHC) on tissue micro arrays in a multi-centric setting. MET gene amplification was defined as MET/centromere ratio≥2 (with average MET GCN≥4), high MET GCN as CGN≥5 and MET IHC positivity (IHC+) as ≥2+ intensity in ≥50% of tumor cells. In addition, 182 MET IHC+ and EGFR/KRAS wild type tumors were analyzed for MET exon-14 skipping mutation. **Results:** MET IHC+ was detected in 23.8% of 2432 patients, and significantly associated with female gender, small tumor size, and adenocarcinoma histology. A high inter-laboratory variability in IHC analysis was observed. MET gene amplification prevailed in 4.6% and MET GCN≥5 in 4.1% of 1572 patients. MET gene amplification and MET GCN≥5 did not associate with any tumor characteristics or stage, but did significantly associate with IHC MET positivity ($p<0.001$). METex14 skipping mutation was detected in 5 of 182 (2.7%) MET IHC+ wild type EGFR/KRAS NSCLC, 4 of which within the 88 adenocarcinomas (4.5%). No association of MET protein overexpression, MET gene amplification or high MET GCN was found with overall survival, recurrence-free survival or time to recurrence. **Conclusions:** MET protein overexpression is detected in 23.8% of resected NSCLC. MET gene amplification is found in 4.6% of tumors and is associated with protein overexpression. Both parameters do not predict prognosis. The large inter-laboratory variability in IHC highlights the challenge of MET IHC analysis in routine practice.

ST47LB. Evaluation of a Promising Molecular Biomarker (CRTC1-MAML2 Rearrangement) Towards Improved Management in Mucoepidermoid Carcinoma (Head & Neck and Thoracic) – Initial Tertiary Care Hospital Experience

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Introduction: Biomarker analysis in a wide range of solid tumours increasingly allows for improved tumour diagnosis, sub-typing, prognostic predictive evaluation and targeted therapy. The current guidelines for reporting salivary gland tumours in the head and neck region (Royal College of Pathologists-RCPath, Nov 2013) highlight only a single molecular biomarker viz., t(11;19)(q21–22;p13) translocation which results in a CRTC1-MAML2 fusion oncogene. This tumour type is not specifically covered in the pulmonary neoplasm document of the RCPath. This marker is currently considered specific to mucoepidermoid carcinomas (MECa) and is proposed as having prognostic significance. This test is not in routine use in the United Kingdom. **Methods:** To perform a feasibility study to establish FISH testing towards identifying MAML2 gene rearrangement in MECa and attempt to correlate its presence/absence with prognostic outcomes. Retrospective identification of surgically treated MECa at Wythenshawe hospital (2010 to 2015) with subsequent block selection followed by FISH probe trial at Christie hospital using Zytolight SPEC MAML2 Dual colour breakapart probe. **Results:** The head and neck tumour group comprised 15 patients (7=MAML2 rearrangement detected; 1= atypical abnormal signal pattern; 5= no evidence of rearrangement; 2 cases = test failed - decalcified tissue). The thoracic tumour group comprised 8 patients (4=MAML2 rearrangement detected; 4 = no evidence of rearrangement). **Conclusions:** Literature supports the benefits of performing FISH for MAML2 rearrangement in MECa for diagnosis and refine prognostic categorisation. In our trial cohort, we have demonstrated the feasibility of identifying MAML2 rearrangement in formalin fixed paraffin embedded tissue. MAML2 rearrangement in MECa identifies a molecular subgroup of patients who may potentially also benefit from targeted therapies - this is the subject of ongoing research.

ST48LB. Multi-site Reproducibility and Analytic Accuracy of the HTG EdgeSeq Immuno-oncology Assay

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Introduction: HTG EdgeSeq assays employ automated qNPA coupled to next generation sequencing to enable multiplex mRNA expression analysis. For this study, data were generated under a standardized protocol that is used for every HTG EdgeSeq system installation. Data from six sites, a mixture of central laboratory and academic genomic labs, have been summarized to demonstrate assay reproducibility and analytic accuracy of the HTG EdgeSeq Immuno-Oncology Assay. The assay comprises probes targeting 549 genes implicated in the host immune

response to tumors. The assay can be used to measure expression levels using a single section of FFPE tissue. All sequencing was performed with Illumina MiSeq instrument in accordance with the manufacturer's instructions. **Methods:** Eight FFPE samples (seven NSCLC and one metastatic melanoma) were lysed at 10mm^2/35µL in triplicate. The lysates were randomized to 24 wells of a 96-well plate and processed and sequenced. We evaluate performance using three criteria: 1. Reproducibility of expression across FFPE samples on a select set of genes (PD-L1 (CD274), PD-1 (PDCD1), PD-L2 (PDCD1LG2), CTLA4, JAK4 (TYK2)); 2. Distribution of mean-variance relationship of expression over the set of 549 genes, as well as summary statistics of triplicate standard deviation (SD) and coefficient of variation (CV); and 3. Agreement between the triplicate samples over all genes. Reproducibility of probe-level expression was qualitatively assessed using Tukey mean-difference (a.k.a. Bland-Altman) plots. Agreement is measured through the concordance correlation coefficient. **Results:** These probes range from lower expression of 5 to 10.5 on the log2 scale (32 to 1448 CPM). There is a high degree of similarity in expression across FFPE samples, with few outliers the Tukey mean-difference for all five selected genes was nearly zero between those processed and sequenced at HTG and those processed and sequenced at the six sites. The mean-variance plots were nearly identical between HTG and sites, with the only differences occurring in very low expressing probes (< 5 $\log_2$(CPM). Average SD between replicates over all eight FFPE samples was between 2.2 to 2.4 across all six sites with a corresponding highest average CV of 2.9, at the lowest level of expression. Average CCC between HTG and the sites was 0.90 over all FFPE replicates; and within FFPE averages (including HTG) was 0.90. **Conclusions:** The HTG EdgeSeq Immuno-Oncology Assay and the HTG EdgeSeq system provides reliable and reproducible gene expression as demonstrated across a total of seven independent sites including HTG.

TECHNICAL TOPICS

TT01. Rapid and Cost-effective Custom Oncology Panels for NEBNext Direct Target Enrichment from Modular, Predesigned Genes

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Introduction: Target-enrichment for next-generation sequencing focuses data collection on user-defined genomic regions of interest for extensive sequencing coverage of the desired regions at a lower cost. Commercially available gene panels that are focused on specific applications or diseases are a cost-effective option for target enrichment, but the specific set of desired genes for any given study often deviates from these readily available panels. More comprehensive panels or whole exome sequencing can ensure inclusion of genes of interest, but results in unnecessary sequencing of irrelevant genomic content and lower depth of coverage of desired regions, reducing sensitivity for low frequency variants within a sample. Thus, customized panels containing only the genes relevant to a study are optimal, however the high cost and lengthy time to design and synthesize a custom gene panel is prohibitive for many genomic research applications. To expand the accessibility of customized gene panels for a wide range of applications, we developed modular baits specific to the full exonic content of 450 cancer-associated genes and validated the ability to combine subsets of these genes into unique, high-performing panels. **Methods:** We prepared a series of custom gene panels from subsets of the 450 predesigned genes. These panels ranged in size from 1 to 100 genes and were used with the NEBNext Direct™ target enrichment protocol and inputs ranging from 10-500ng of intact or FFPE-degraded DNA. The resulting Illumina-compatible libraries were sequenced on the MiSeq platform to determine the sensitivity of variant detection across DNA input amounts and target panel sizes. **Results:** All libraries were successfully prepared in one day, sequenced overnight, and analyzed the next day, resulting in a two-day turnaround time. The coverage uniformity of the same gene present in a variety of custom panels remained constant, indicating predictable performance regardless of the identity or quantity of additional genes in the pool. The specificity of capture for the custom panels was typically greater than 90% and independent of panel size. However, specificity was reduced if the panel included genes that have known pseudogenes. As expected, sensitivity was greater with small panels and/or larger DNA input amounts, with variants detected at as low as 1% allelic frequency. **Conclusions:** Unique panels generated from subsets of our 450 predesigned, cancer-associated genes display predictable capture performance with high specificity, coverage uniformity, and sensitivity across a wide range of panel sizes. Thus, we can rapidly produce cost-effective, highly scalable, custom gene panels to target specific genes for a wide range of genomic research and diagnostic applications.

TT02. Comprehensive Detection of All Major Classes of MET Deregulation by Anchored Multiplex PCR and Next-Generation Sequencing

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Introduction: Deregulation of the proto-oncogene, MET, confers an aggressive phenotype in a variety of human cancers, promoting proliferation, invasive growth and

angiogenesis. MET deregulation can be driven by gene amplification, overexpression, exon 14 skipping, gene fusions and single nucleotide variants (SNVs), such as kinase-activating point mutations. MET is a target of intensive drug development efforts, although the various mutated forms of MET exhibit unique drug sensitivities. Therefore, detection of these mutations has an important role in the development of drugs targeting MET, and has the potential to guide treatments for cancers driven by MET deregulation. We developed a targeted NGS assay based on Anchored Multiplex PCR (AMP™) to detect all types of mutations driving MET deregulation from a single sample. **Methods:** AMP only requires a single gene-specific primer for amplification, enabling open-ended capture of DNA and cDNA fragments for NGS-based detection of known and unknown mutations. We developed AMP-based Archer® VariantPlex™ and FusionPlex® library preparation assays to detect mutations from DNA and RNA, respectively. AMP probes were designed to cover the MET gene for detection of copy numbers variants (CNVs) and SNVs from DNA, and known and novel fusions, exon skipping and expression levels from RNA. **Results:** We show that the VariantPlex assay enables NGS-based detection of MET amplifications from DNA in concordance with FISH results. Further NGS analysis of RNA from the same sample using the FusionPlex assay revealed the resulting overexpression of MET. We also demonstrate that AMP-enabled open-ended capture of cDNA fragments allows for reliable detection of exon 14 skipping in FFPE samples and in cells, consistent with RT-PCR results. Parallel analysis of DNA from the cell samples revealed splice site mutations that have been previously reported to drive exon 14 skipping. Furthermore, this open-ended capture also permitted identification of a novel GTF2I:MET gene fusion in a patient-derived xenograft model. Finally, we detected a kinase-activating point mutation in MET, p.Y1253D, by analysis of genomic DNA with the VariantPlex NGS assay. **Conclusions:** These results show that AMP-based VariantPlex and FusionPlex Assays enable comprehensive detection of multiple mutation types from low-input clinical sample types, such as FFPE specimens. As MET deregulation can be driven by many different genetic aberrations, this allows for NGS-based characterization of MET deregulation from a single sample.

TT03. Rapid and Comprehensive Detection of CFTR Variants Across Ethnic Groups using Anchored Multiplex PCR and Next-Generation Sequencing

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Introduction: Cystic Fibrosis (CF) is an autosomal recessive disease caused by mutations in the CF transmembrane conductance regulator (CFTR) gene. CF is characterized by the build-up of thick mucus resulting in chronic lung infections and airway inflammation. Carrier identification and newborn screening have significant implications in the overall prognosis of CF patients. Underlying CFTR mutations were recently shown to vary significantly across ethnic groups. However, current CFTR genotyping assays detect mutations highly prevalent in white individuals, yet fail to detect mutations that are more prevalent in nonwhite individuals. We present a rapid, cost-effective assay for comprehensive detection of CFTR mutations for pan-ethnic carrier identification and newborn screening. **Methods:** BabyGenes, Inc, in partnership with ArcherDX, Inc., has developed a targeted next-generation sequencing (NGS) assay based on Anchored Multiplex PCR (AMP™) to detect mutations in 105 genes clinically linked to inborn errors in metabolism, including CFTR. AMP is a library preparation method for NGS that uses unidirectional gene-specific primers (GSPs) and molecular barcoded adaptors ligated to random start sites to enrich for both known and unknown mutations across a panel of target regions. Following analytical and clinical validation of the panel, a total of 1,585 clinical samples representing a diverse ethnic set were analyzed for clinically significant CFTR variants. **Results:** Clinical validation with 150 blinded specimens from the Coriell Institute for Medical Research resulted in 100% accuracy of variant detection within the CFTR gene. Pan-ethnic screening of 1,585 clinical samples identified 34 unique mutations, several of which were identified in multiple individuals. 73% (25/34) of these unique mutations and 60% (74/123) of total mutations detected are not currently included in the ACMG-recommended 23-mutation panel for CF carrier screening. Furthermore, this screening revealed ethnic differences in clinically significant CFTR variants and a pan-ethnic carrier rate of approximately 7%. **Conclusions:** We demonstrate that the BabyGenes, Inc. AMP-based targeted NGS assay enables rapid, highly sensitive, and comprehensive detection of both known and novel mutations in the CFTR gene. This is critical for global carrier and newborn screening, as CF driver mutations have not been fully characterized across all ethnicities. Findings suggest that the pan-ethnic carrier rate of CF may be higher than originally predicted. As this entire assay can be performed in under 96 hours and the reagents do not require refrigeration, AMP is a practical and economical method for global communities.

TT04. Anchored Multiplex PCR Enables Sensitive and Specific Detection of Variants in Circulating Tumor DNA by Next-Generation Sequencing

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Introduction: Liquid biopsies are a promising, minimally invasive alternative to tissue biopsies that have potential

cost, time and safety benefits, as well as a greater ability to interrogate heterogeneous tumors. However, except in advanced disease states, cell free DNA (cfDNA) is typically of low abundance and only a small portion of cfDNA originates from tumor cells as circulating tumor DNA (ctDNA), which tends to be highly fragmented (100-300bp). Therefore, NGS-based assays to detect variants in ctDNA must be sensitive enough to detect mutations at allele frequencies (AF) <2% from <100ng of highly fragmented DNA. **Methods:** We developed the Archer® Reveal ctDNA™ 28 assay based on Anchored Multiplex PCR (AMP™), a target enrichment method for NGS that uses unidirectional gene-specific primers and molecular barcoded (MBC) adapters for amplification. AMP is well suited to amplify small cfDNA fragments, as it only requires one intact primer-binding site within a fragment. Single primers capture target regions from both strands independently, increasing the sensitivity of variant detection from low-input samples. MBC adapters ligated prior to amplification permit post-sequencing error correction, reducing background noise and increasing analytical sensitivity of ultra low-allele frequency variant detection. Finally, variant filtering in the Archer Analysis pipeline further increases the specificity of variant calls. **Results:** Using commercially available reference ctDNA standards, we demonstrate that genomic DNA present in plasma does not significantly impact amplification of small, fragmented ctDNA with the AMP-based Reveal ctDNA 28 assay. Based on sequenced reads, AMP enabled interrogation of more than 65% of the input molecules from 50ng starting material. As a result, we show 100% detection sensitivity for 1% AF variants using 10ng DNA input and 71.9% detection sensitivity for 0.1% AF variants using 50ng DNA input. MBC-enabled post-sequencing error correction and variant filtering reduced the number of false positives by 98%, resulting in 91.7% specificity. Finally, mutations detected from liquid biopsy-derived ctDNA showed cancer type-dependent concordance with tissue biopsy findings, and revealed additional oncogenic driver mutations. **Conclusions:** The Archer Reveal ctDNA 28 assay is a powerful tool for sensitive and specific NGS-based detection of variants in ctDNA, demonstrating accurate allele frequency quantification of synthetic reference standards. This assay is a promising approach to characterize solid tumors from liquid biopsies, showing cancer type-dependent concordance of tissue and plasma mutation profiles, as well as identification of additional oncogenic driver mutations in ctDNA.

TT05. Characterization of Hematologic Malignancies with Anchored Multiplex PCR and Next-Generation Sequencing

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Introduction: Hematologic malignancies can be driven by a diversity of mutation types, including single nucleotide variants (SNVs), copy number variants (CNVs), gene fusions, insertions and deletions (indels) and changes in gene expression profiles. However, comprehensive detection of these mutation types from a single clinical sample is challenging, as specific assays are required to detect each mutation type. We developed targeted next-generation sequencing (NGS) assays based on Anchored Multiplex PCR (AMP™) to simultaneously detect multiple mutation types, including but not limited to novel gene fusions and ITDs, as well as relative gene expression levels relevant in hematologic malignancies.
Methods: AMP is a library preparation method for NGS that uses molecular barcoded (MBC) adapters and single gene-specific primers (GSPs) for amplification. We developed AMP-based Archer® VariantPlex™ and FusionPlex® assays for NGS-based detection of mutations from DNA and RNA, respectively. Open-ended amplification permits identification of both known and novel gene fusions with FusionPlex assays. Furthermore, we developed a VariantPlex assay and novel bioinformatics algorithm to detect ITDs from clinical DNA samples. Finally, MBC adapters ligated to RNA fragments prior to amplification enable determination of relative gene expression levels. **Results:** We show that open-ended amplification from KMT2A GSPs enabled detection of a KMT2A-MLLT3 fusion through breakpoint identification, with reads extending 6 exons into MLLT3. AMP also enabled NGS-based detection of a novel RUNX1 fusion, RUNX1-G6PD, in a case of acute unclassifiable leukemia. Furthermore, we show that single, unidirectional GSPs provide bidirectional coverage of a BCR-ABL1 fusion, which was detected with reads originating from ABL1 as well as BCR GSPs. Using our optimized bioinformatics algorithm and the VariantPlex assay, we accurately and reliably detected ITDs of varying sizes and insertion points, with simultaneous point mutation detection in AML-positive blood samples. Finally, we show NGS-based expression profile analysis with the FusionPlex assay, resulting in identification of Diffuse Large B-Cell Lymphoma subtypes in a small cohort of samples. **Conclusions:** Our results demonstrate that AMP-based NGS enables comprehensive detection of multiple mutation types as well as gene expression levels relevant in hematologic malignancies. Importantly, AMP enables identification of known and novel gene fusions at nucleotide resolution, detection of ITDs and characterization of relative gene expression levels.

TT06. B- and T-cell Immune Repertoire Characterization by Anchored Multiplex PCR and Next-Generation Sequencing

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Introduction: The adaptive immune system is involved in various disease conditions including cancer, chronic infection, autoimmune disease and transplant rejection. Adaptive immunity is mediated by B and T lymphocytes, which are activated upon antigen binding to antigen receptors expressed on their surface. Therefore, the spectrum of these antigen receptors, or immune repertoire (IR), provides a means to monitor adaptive immune responses to disease, vaccination and therapeutic interventions. Next-generation sequencing (NGS) of antigen receptor genes is a valuable tool in the study of disease states and responses to various interventions. Traditional amplicon-based NGS assays use opposing primers for targeted amplification of rearranged antigen receptor genes. Thus, large primer panels are required to capture the extensive combinatorial diversity exhibited by the IR. Quantification from such assays requires a complex system of synthetic controls to account for differential amplification efficiency across segment combinations. Here, we describe an Anchored Multiplex PCR (AMP™)-based NGS assay to analyze the IR, employing a minimal set of gene-specific primers in conjunction with molecular barcodes (MBCs) to reduce amplification bias.
Methods: AMP uses MBCs ligated to cDNA ends and gene-specific primers for amplification, enabling immune chain mRNA interrogation from a single side. This eliminates the need for opposing primers that bind within the highly variable V-segment, eliminating clone dropout due to somatic hypermutation. Furthermore, this facilitates CDR3 sequence capture from highly fragmented RNA inputs. **Results:** We validated the quantitative reproducibility and sensitivity of the AMP-based IGH assay using mRNA isolated from peripheral blood leukocytes of healthy and B-cell chronic lymphocytic leukemia (B-CLL) donors. Our data showed high reproducibility between replicates and quantitative clone tracking down to 0.01%, with the ability to determine IGHV mutational status. We also validated the quantitative reproducibility and sensitivity of the AMP-based T-cell receptor (TCR) assay using high-quality mRNA isolated from peripheral blood leukocytes and highly fragmented RNA isolated from formalin-fixed paraffin-embedded (FFPE) samples. Our data indicate that clonal diversity in sequencing data is driven by input quantity, total T-cell number, and, to a lesser degree, mRNA quality. **Conclusions:** AMP-based NGS with MBC quantification and error-correction is a powerful method to characterize the immune repertoire.

TT07. A Targeted Resequencing Approach to Identify Actionable Somatic Copy Number Alterations with High Sensitivity Alongside SNVs and Indels from Clinical Tumor Specimens

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Introduction: Targeted, next generation sequencing is increasingly used to identify actionable mutations in cancer patient samples. However, the typical specimen available is formalin-fixed, paraffin-embedded (FFPE) blocks, which yield low quantity of damaged DNA. Inability to completely repair the ends of damaged DNA reduces the yield of adapter ligation in double stranded enrichment methods. This ensues the need for PCR amplification prior to target capture which, in turn, introduces PCR biases that impairs the sensitivity for detecting of copy number alterations (CNAs), an important biomarker for targeted therapy. In short, there is a need for a library preparation platform that performs well with low quality and quantity DNA without relying on massive PCR amplification. **Methods:** We present a single-stranded enrichment assay that removes the need of double-stranded template repair and can be utilized without pre-capture PCR on small amounts of damaged and fragmented DNA from FFPE blocks. Damaged bases are removed by excision and afterwards DNA is melted and ligated to single stranded universal adaptors. Capture is then performed by annealing and extension of short target-specific oligonucleotide probes designed to tile densely across regions of interest in both strands. A final expansion of the library to load into the Illumina sequencer is performed by a few cycles of post-capture PCR. This protocol can be achieved in a single day. Our standard oligo-probe mixture targets the exons of 131 cancer genes. We analyze the data with our own tumor-only SNV and CNA identification algorithms.
Results: We sequenced libraries from reference and cancer cell lines with reported CNAs, reference materials from Horizon Diagnostics, and clinical FFPE samples, in the Illumina NextSeq in sets of 12-24 samples. Our sequencing metrics show on-target rates of 60-80% and high uniformity, as evidenced by Fold 80-base Penalty metrics of <2.5, and coverage mean depths of >3,000X, regardless whether the sample is a cell line or clinical FFPE, and for DNA inputs ranging from 10-100ng. Using 11bp random molecular barcodes inserted in the universal adaptor we demonstrate a remarkably low duplicate rate (median copies=1). Importantly, we show a high concordance between the copy number detected with our assay and the orthogonal ddPCR validation data in cell lines harboring CNAs, ranging from 0 to 16 copies, with an r2 of 0.96. Further, SNV/indel detection sensitivity and specificity in reference materials (SeraCare STMM) is a par with CNA performance. **Conclusions:** Our library preparation method that relies on single stranded adapter ligation and anneal-extend target capture generates uniform coverage with minimal PCR requirement resulting in highly sensitive CNA calling.

TT08. Detection of Hotspot Cancer Mutations in a Clinical Laboratory Using Next Generation Sequencing on the Ion Torrent PGM

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Introduction: Recent advances in sequencing technologies have enabled us to scrutinize the versatile underlying mechanisms of cancer more precisely. However, adopting these new sophisticated technologies is challenging for clinical labs as it involves complex workflows, and requires validation for diagnostic purposes. The aim of this work is towards the analytical validation of a next generation sequencing (NGS) method for cancer hotspot mutation analysis. **Methods:** Characterized formalin-fixed paraffin-embedded (FFPE) samples including biopsy specimens and cell-lines were purchased. DNA was extracted from FFPE biopsy sections or similarly prepared reference standard cell lines. After quantification of extracted DNA, multiplex PCR amplification of target regions was performed followed by library preparation, template preparation and sequencing on the Ion Torrent PGM platform using the Ion PGM Select Sequencing Kit and Ion 318 chip. Torrent Suite software version 4.4.3 and Ion Reporter Analysis software v4.2 were employed for sequence analysis. **Results:** The most important parameters that should be determined in the validation of any NGS assay for a clinical laboratory are coverage, threshold (cutoff point for differentiation of a positive and a negative result), sensitivity, specificity, and limit of detection (LoD). The minimum coverage and threshold are the highest priority parameters because they affect all others. To determine the most appropriate coverage, a difference plot was constructed showing coverage on the X-axis versus measurement-error (the deviation of the measured frequency from the expected frequency) on the Y-axis. Variant frequency calls with coverage of <100x were found to be inaccurate. Receiver operating characteristics (ROC) analysis showed the most appropriate threshold to be 2%. To determine LoD, logistic regression analysis was performed. Accordingly, the sensitivity, specificity and limit of detection of the method were of 96.1%, 97.8% and 4.3% respectively. **Conclusions:** In every validation study, the number of samples, the manner of sample selection, and the number and type of variants play a role in the outcome. The range of minimum acceptable coverage varies from 100x to 500x, and different thresholds typically from 2 to 8 percent. According to our results, the Oncomine Focus Assay performs within parameters reported by other laboratories with an acceptable limit of detection and accuracy. Lastly, the type, number and frequency of mutations are to be considered in the light of clinical actionability and outcome when conducting a validations study. Therefore, these parameters should be evaluated according to the clinical needs of each laboratory undertaking the validation.

TT09. Implementation of a Comprehensive NGS Workflow as an Integrated Solution for Clinical Cancer Diagnosis

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Introduction: In recent years, rapid advances in cancer genetics and targeted therapy have made "personalized cancer care" a possibility. These advances are largely aided by clinical adoption of Next Generation Sequencing (NGS) and the advance of targeted therapies. Our institution, Legacy Health, is a community-based five hospital network striving to provide best-in-class healthcare to the Portland metropolitan region. As such, our molecular diagnostics laboratory sought to implement an NGS workflow to enable tumor molecular diagnosis and precision cancer care. **Methods**: Following thorough comparison of both performance and bioinformatics pipeline in different NGS platforms, we decided to implement the fully integrated GeneReader workflow (QIAGEN). This system includes all hardware, software and reagents needed to analyze FFPE and liquid biopsy samples, and generate a clinically actionable reports that are both "necessary and sufficient" for NGS testing of the five most common cancers in the US population. Sixty samples from patients with cancers of the breast, ovarian, colon, lung, and melanoma and previously tested with either a send-out service or onsite PCR were used for test validation and system verification. **Results:** We observed a high degree of concordance in variant identification between the GeneReader NGS system and alternative tests. In addition, we were able to demonstrate satisfactory system performance in sample custody, QC metrics, sequencing coverage and variant interpretation. We also explored ways to improve the time and process for workflow integration and test validation. These efforts enabled our laboratory to adopt NGS as a Lab Developed Test (LDT) in a CLIA-approved and CAP-compliant environment. As a result, our network hospitals and physicians can now access NGS testing in a locally based, operationally efficient and economically sustainable manner. **Conclusions:** An inherently complex process, clinical adoption of NGS technology still represents a major challenge to many laboratories. As 80% of cancer patients are treated in community settings, the implementation of this technology by local hospitals is critical to the successful implementation of precision cancer care. Our hospital has identified the GeneReader System to be a feasible solution, and our experience can be replicated by others desiring to adopt NGS with limited resources.

TT10. A Full Process Control for Setting up an NGS Operation Using the GeneReader System

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Introduction: NGS has proven to be a powerful tool for cancer research, enabling insights into variants involved in tumor evolution and with implications for personalized disease management. However, adoption of NGS by new laboratories is challenging as it requires custom adoption of every individual step of a complex yet fragmented workflow, from DNA or RNA extraction to variant detection and interpretation. Furthermore, any laboratory desiring to adopt NGS face the challenge of verifying the entire workflow including all components, as well as establishing quality control measures as part of routine practice. QIAGEN's GeneReader NGS System offers the first true NGS Sample-to-Insight workflow, solving this great unmet need. All building blocks required to enable NGS workflow have been assembled and optimized to deliver actionable cancer insights. Furthermore, this workflow has now been verified by a full process control study. **Methods:** FFPE reference standards and clinical samples from Horizon were used. DNA extractions were conducted using the QIAGEN GeneRead DNA FFPE Kit and the DNA was then processed through the GeneReader NGS workflow using the GeneRead QIAact Actionable Insights Tumor Panel for targeted PCR. The QIAGEN Clinical Insight Analyze (QCI-A) and QCI-Interpret (QCI-I) bioinformatics pipelines were used for analysis and interpretation of results. Results were cross-compared to known variant allele frequencies (VAFs) of these samples. Robustness and reproducibility of the system was also tested through repeat workflow runs under different conditions. **Results:** High degree of uniform coverage was achieved in this study, with a <4 fold difference between the highest (10,000x) and lowest (2,800x) reads. In all cases sample testing with the GeneReader NGS System and GeneRead QIAact Actionable Insights Tumor Panel correctly identified the known variants, with a minimal allelic frequency of 5%. These variants included single nucleotide variants (SNVs) and insertion-deletion variants (InDels). When standard laboratory operating conditions were changed, including sample pooling, reagent batch, operator experience level and individual system utilization, the GeneReader continued to yield consistent results, with <10% variability in VAF. **Conclusions:** This study demonstrates the power of optimizing an entire workflow, and integrating verification process as part of the overall NGS solution. The consistency in performance makes the GeneReader System an ideal option for laboratories interested in implementing NGS technology, but without the deep experience or resources in customizing a full solution. The data presented also provides an invaluable resource in guiding the setup, testing and troubleshooting of an NGS operation.

TT11. Comparison of custom designed gene panels for hybrid capture-based parallel sequencing in molecular pathology routine diagnostics

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Introduction: In recent years, parallel sequencing technologies have become increasingly integrated into daily clinical practice. Already, many institutions use amplicon-based parallel sequencing approaches for the analysis of multiple targetable genes. However, amplicon-based parallel sequencing assays do not routinely detect chromosomal aberrations or copy number changes. Hence, these are still widely analysed by FISH and IHC. The development of new technologies to detect all therapeutically relevant genomic alterations in a single assay is an ongoing process. On DNA level, hybrid capture-based parallel sequencing is used for the simultaneous detection of somatic gene mutations, gene fusions and copy number alterations. This study aims to evaluate and compare custom hybrid capture-based panels and library preparation protocols from 2 different leading suppliers and the transfer into routine diagnostics. **Methods:** In this approach, DNA extracted from formalin-fixed, paraffin-embedded (FFPE) tissue is sheared and libraries are prepared and pooled. Two custom hybrid capture-based panels are designed covering the same target regions of 83 genes. The custom designed, biotinylated DNA probes are hybridized to target sequences to allow for sequence enrichment using streptavidin beads. Finally, libraries containing the target sequences are sequenced on platforms NextSeq from Illumina (San Diego, CA). The performance of the 2 custom panels from each supplier as well as the sequencing results were compared on FFPE material. **Results:** Differences in the number of amplicons of the designed custom panels were seen between the two suppliers as well as the library preparation workflow. Data analysis revealed variations in the distribution of reads on sequenced probes, the coverage uniformity, the number of PCR duplicates and the targeted aligned reads. At this point, only samples with good DNA quality and quantity have been selected to ensure the assay is feasible. **Conclusions:** This study showed that the design of custom hybrid capture-based panels varies between companies. Protocols and custom panels may require optimisation for FFPE material and the needs of a routine diagnostics laboratory. A Major challenge is the bioinformatics, especially the detection of gene fusions and copy number changes. Data analysis is extremely complicated and time-consuming, and custom data analysis pipelines and experienced bioinformaticians are required. Additionally, our experience indicates that DNA

extraction from up to 20% of lung cancer samples will yield <50 ng DNA, due to limited tissue from small biopsies. Thus, using hybrid capture-based technologies will necessitate concurrent use of amplicon-based methods for smaller samples, unless methods are improved.

TT12. Liquid Biopsy: EGFR Mutation Testing Using Cell-free DNA and the GeneReader Workflow in a Cohort of Patients with Advanced EGFR-mutant NSCLC

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Introduction: Non-small cell lung cancer (NSCLC) is one of the most common types of cancer worldwide, which internationally accounts for over a million deaths every year. By now tyrosine kinase inhibitors (TKIs), which target the epidermal growth factor receptor (EGFR), have become an alternative treatment option for patients with NSCLC. According to many studies it is evident that activating *EGFR* gene mutations are effective markers for EGFR-TKIs sensitivity. Reliable analysis of such mutations has previously been possible only in tumor tissue. Now the isolation of cfDNA (circulating cell-free DNA) from blood samples, the so called liquid biopsy, is an alternate and minimally invasive way of extracting DNA for mutation analysis. This approach even enables the analysis of tumor changes in nearly real-time. The aim of this study was to evaluate the presence of *EGFR* mutations in cfDNA in a cohort of patients with advanced *EGFR*-mutant NSCLC using the Qiagen GeneReader workflow. **Methods:** We evaluated the *EGFR* mutation status in cfDNA from 13 NSCLC patients with known primary *EGFR* mutations before or during TKI treatment using the Actionable Insights Tumor Panel and the Qiagen GeneReader workflow. **Results:** In 6/13 cases the primary activating *EGFR* mutation could be detected in cfDNA with an allele frequency ranging from 0.59% to 8.48% (mean. 3.69%; std. dev. 0.0288). In cases with positive detection of *EGFR* mutations in cfDNA, there was 100% concordance with the specific *EGFR* mutation as determined previously in FFPE biopsies. Additionally, a secondary *EGFR* T790M mutation with an allele frequency of 1.81% could be detected in one patient, indicating a resistance to TKI therapy and the need for therapy adjustment. Indeed, this patient received a 3rd generation EGFR TKI and showed good partial response. **Conclusions:** We could fabricate a proof of concept for the detection of *EGFR* mutation by massively parallel sequencing of cfDNA. Our results suggest that this approach is of adequate diagnostic accuracy. cfDNA analysis by sequencing should be a valuable tool for analysis and detection of therapeutically relevant mutations. In our study, the GeneReader cfDNA workflow showed a sufficient sensitivity for further studies in the field of cfDNA diagnostics.

TT13. Multi Institutional Evaluation of a High Sensitive NGS Assay for Liquid Biopsy Mutation Detection in Lung Cancer

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Introduction: The detection of actionable mutations in lung cancer is still a major challenge due to the lack of tissue specimens for molecular profiling of the tumor in approximately 25% of lung cancer patients. The cell-free (cf) circulating tumor DNA (ctDNA) isolated from plasma of cancer patients is an alternative, minimally invasive source of tumor DNA that allows multiple determination of the mutational tumor status over time. However, the intrinsic low abundance of ctDNA makes the mutation detection and its quantification in plasma a challenging task. Here we report a multi-institutional validation of the Oncomine cfDNA Lung assay for the analyses of cfDNA in molecular diagnostics laboratories. **Methods:** The Oncomine cfDNA Lung assay is a multiplexed sequencing assay using molecular barcodes to tag each original DNA molecule used as input material. This enables the highly sensitive, quantitative and reproducible detection of low frequency mutations providing an ideal assay for liquid biopsies. To ensure an unbiased and uniform evaluation of the assay, the Horizon DX cfDNA reference standard derived from human cell lines, fragmented to an average size of 160bp to closely resemble cfDNA extracted from human plasma, was employed. This reference standard covered eight mutations in *EGFR, KRAS, NRAS* and *PIK3CA* at 5%, 1%, and 0.1% allele frequency and the wildtype allele. The same batch of control samples was distributed to the participating laboratories within the OncoNetwork Consortium. Samples were sequenced twice in each laboratory either using the Ion PGM or the Ion S5 system. Libraries were templated using the Ion Chef and multiplexed according to the sequencing system as 4 libraries on a 318/520 chip or 8 libraries on a 530 chip. A

bioinformatics pipeline within the Torrent Server software allowed for automated variant calling. **Results:** The laboratories involved in the study were able to detect all eight hotspot base changes and indels present in the reference samples at allele frequencies from 0.1% to 5% with an average of 94.05% sensitivity (range 87.50-97.92%) and an average of 99.87% specificity (range 99.53-100%). When only considering variants at the 0.1% allele frequency, the average sensitivity was 83.04% (range 68.75-99.95%) and the average specificity was 99.95% (range 99.68-99.95%). Notably, at 0.1% allele frequency, all laboratories accomplished to detect the challenging *EGFR* p.T790M variant, which is a marker of sensitivity to EGFR tyrosine kinase inhibitors. **Conclusions:** These preliminary data confirm the potential of the Oncomine cfDNA lung assay for plasma genotyping, which allows for the noninvasive, multiplexed, sensitive and reliable detection of complex, targetable genomic alterations in lung cancer.

TT14. EGFR Mutation Analysis in ct-DNA From NSCLC Patients

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Introduction: Lung cancer is the most commonly diagnosed cancer and the leading cause of cancer-related deaths. Non-Small-Cell Lung Cancer (NSCLC) accounts for approximately 83% of all lung cancers. Tyrosine kinase inhibitors of epidermal growth factor receptor (EGFR-TKIs) are considered as effective treatments for EGFR mutated NSCLC patients although response to 1st and 2nd generation TKIs is limited due to the emergence of resistance mutations such as p.Thr790Met. In these cases 3rd generation TKIs could be administered. EGFR mutation analysis in circulating cell free-tumor DNA (ct-DNA) from plasma constitutes a convenient and less invasive alternative method in cases without adequate tumor material for molecular analysis or monitoring of progression. **Methods:** We analyzed 61 blood samples of which, 28 had matched paraffin-embedded tumor tissues for EGFR mutations from patients with NSCLC. Circulating cell free-tumor DNA (ct-DNA) isolation from plasma and detection of EGFR mutations was performed using Cobas® EGFR mutation test v2. **Results:** EGFR mutations in ct-DNA were detected in 12 cases (19.7%) and in 3 cases (25%) double mutations were identified. The samples with double mutations displayed a resistance mutation, p.Thr790Met along with a sensitizing mutation; p.Leu858Arg in 2 cases or a deletion in exon 19. The most common EGFR mutations were exon 19 deletions (50%), followed by exon 21 point mutation p.Leu858Arg (42%). In one sample, p.Leu861Gln was detected (8%). The analysis of 28 ct-DNA samples from plasma versus matched tumor DNA showed 73% sensitivity, 100% specificity, 100% PPV, 81% MPV, for the detection of EGFR mutations. The concordance of EGFR testing between the two sampling methods was 86%. Notably, the discordant negative ct-DNA samples displayed exon 18 mutations in 2 cases, exon 21 point mutation p.Leu861Gln in one case and an exon 19 deletion in 1 case. **Conclusions:** The above analysis adds to current data indicating that EGFR mutation testing in ct-DNA has high specificity and positive predictive value. Although, its sensitivity is lower in comparison to EGFR mutation analysis in tissue samples due to limiting factors (amount of ctDNA in the plasma, efficiency of DNA extraction, sensitivity of mutation analysis methods), still it represents a promising alternative for patients with insufficient/low quality tissue. Concluding, EGFR mutation-positive cases by a ctDNA test can be reported with certainty, whereas for patients with an EGFR mutation-negative ctDNA test, a biopsy should be obtained ideally in order to ascertain an accurate mutation status.

TT15. Crude Lysates from FFPE Tissues are Well Suited for Copy Number Analysis by a Novel Digital MLPA Assay

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Introduction: Formalin-Fixed Paraffin-Embedded (FFPE) tissue-derived genomic DNA has been reliably used for analysis of gene copy number status by Multiplex Ligation-dependent Probe Amplification (MLPA) in a large number of studies. Although MLPA is not hindered by DNA fragmentation of FFPE samples, formalin-induced DNA crosslinking and base modifications can affect MLPA if not eliminated or reduced during DNA extraction. In this study various DNA extraction methods were compared in terms of in suitability for a novel digital MLPA (dMLPA) assay. dMLPA assay is based on the well-known MLPA procedure but can include up to 1000 probes in a single reaction, uses minimal amounts of DNA (≥20ng) and can be analysed on all Illumina NGS platforms. **Methods:** Four commercial kits (RecoverAll Total Nucleic Acid Isolation kit, QIAamp DNA FFPE tissue kit, Zymo Research FFPE DNA miniprep, WaxFree DNA extraction kit) and 1 in-house method (producing crude tissue lysates) were used to extract genomic DNA from 7 different healthy FFPE tissue types. For dMLPA, a probe set of about 500 probes targeting various locations on all chromosomes was analysed in one reaction. After probe hybridisation, ligation and PCR steps, PCR products were loaded onto an Illumina MiSeq sequencer. FastQ output files were analysed in-house and data were normalized against reference samples (commercial genomic DNA). **Results:** The highest percentage of normal copy number ratios on

the DNA samples extracted using the in-house method (86-100%), followed by the WaxFree DNA extraction kit (74-99%), Zymo Research FFPE DNA miniprep kit (68-98%), QIAamp DNA FFPE Tissue kit (49-87%) and the RecoverAll Total Nucleic Acid Isolation kit (27-70%). Copy number ratios also varied depending on the tissue type: FFPE tissue DNA samples from kidney had the highest and brain the lowest percentage of MLPA probes with normal copy number ratios regardless of the DNA extraction method chosen. **Conclusions:** Our study demonstrates that FFPE-derived DNA is well-suited for copy number analysis of 500 probes in a single reaction by dMLPA assay. Moreover, as crude cell lysates contain genomic DNA of sufficient quality for dMLPA, the use of commercial FFPE tissue DNA extraction kits might be superfluous. Minor variations in copy number ratios due to the DNA extraction conditions will be reduced when reference samples are of the same tissue and DNA is extracted with the same method as the test samples. To gain insight on the suitability of minute amounts of FFPE derived DNA for dMLPA, a titration experiment with crude tissue lysates will be performed, and also FFPE derived patient DNA with confirmed copy number alterations will be analysed with dMLPA and these results will be presented at the meeting.

TT16. Molecular Analysis of Circulating Free DNA (cfDNA) From Lung Cancer Patients in Routine Laboratory Practice: A Cross-platform Comparison of Three Different Molecular Methods for Mutation Detection

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Introduction: Cell free DNA (cfDNA), which is isolated from blood plasma, represents a noninvasive material for the detection of mutations conferring resistance against EGFR tyrosine kinase inhibitors in non-small cell lung cancer (NSCLC) patients. In advanced disease stages, taking regular biopsies is often not possible because of the general health condition of the patients. Furthermore, a biopsy of a single tumor lesion or metastasis may not reflect the heterogeneous genotype of the tumor and its metastases. To address these issues, cfDNA represents an alternative material for molecular monitoring of patients under therapy. **Methods:** Three molecular approaches (digital PCR, NGS and qPCR) to detect clinically relevant *EGFR* mutations in cfDNA were validated with commercially available cfDNA reference material including known pathogenic mutations (5, 1, and 0.1% mutation frequency, respectively). Further, we analyzed 40 cfDNA preparations from patients with lung cancer to compare reliability and sensitivity of the 3 methods under routine conditions. **Results:** Digital PCR and NGS both detect reliably 0.1% allele frequency in the reference material. A limit of detection for mutation calling in digital PCR (5 counts) and NGS (20 reads) could be established. Only 1 patient showed inconsistent results in *EGFR* mutation profiling in the comparison of the 3 molecular approaches. In total, 65.4% known primary *EGFR* mutations could successfully be detected in cfDNA. In 58.8% of the patients with detectable *EGFR* primary mutations, we could identify a p.T790M mutation which is known to cause resistance against first-line EGFR tyrosine kinase inhibitor therapy (TKI). **Conclusions:** Analyzing cfDNA from lung cancer patients can be a useful tool for testing lung cancer patients under TKI therapy for upcoming resistance mutations. But, limited amount of cfDNA and unknown DNA content from the tumor include a high risk of false-negative results, even with sensitive and well-validated molecular detection methods. Therefore, cfDNA analysis can support, but not replace tissue biopsy testing completely. For patients with known *EGFR* primary mutation under TKI therapy, analysis of cfDNA is a non-invasive alternative for regular testing, with high sensitivity to detect upcoming *EGFR* resistance mutations. If tumor cfDNA is present, which can be confirmed by detecting the primary *EGFR* mutation, p.T790M mutation analysis can be performed with high sensitivity down to 0.1% in the blood plasma.

TT17. Analysis of SHOX2 / PTGER4 DNA Methylation for Lung Cancer Detection in Liquid Biopsies is Robust against Specific Variations in Blood Collection, Transport, and Storage Conditions

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Introduction: Aberrant DNA methylation detected in cell free DNA from liquid biopsies has been demonstrated to be clinically useful for detection of colorectal and lung cancer in asymptomatic patients.[1,2] Availability of IVD kits specifically developed for extraction and bisulfite conversion of plasma-derived DNA supports the standardization and reliability of the general approach. In a similar manner the impact of specimen collection, transport and storage needs to be controlled in order to achieve reliable test results. Here, we report on the robustness of the SHOX2 / PTGER4 DNA methylation panel workflow against specific variations in specimen logistics.
Methods: Whole blood specimens were collected in pairs with collection tubes from various vendors (Becton Dickinson, Qiagen, Roche, Sarstedt, Streck) from up to 12 healthy blood donors. For each pair 1 specimen was spiked with DNA that is target for the methylation assays. Then, pairs of specimens were kept at storage conditions supported by vendor's instructions for use including extended storage at ambient temperature. Thereafter, plasma was prepared and processed according to instructions of SHOX2 / PTGER4 DNA methylation panel workflow. Assay results were compared against a standard protocol for blood collection in a K2EDTA tube with

immediate plasma preparation. **Results:** Assay results did not differ significantly when collection tubes containing different anticoagulants were compared as well as when whole blood specimens were kept at cooled conditions for limited time (< 24 hours). However, non-significant impact on the assay results of extended storage time (>24 hours) of whole blood specimens at ambient temperature was observed only for those collection tubes that were specifically developed for preservation of cell free DNA. **Conclusions:** The SHOX2/PTGER4 DNA methylation panel workflow is compatible with a broad range of available blood collection tubes. Use of specialized blood collection tubes facilitates access to liquid biopsy analysis for a broader population while high reliability is maintained via centralized testing.

References:

1. US Food and Drug Administration. Letter confirming approval of application for premarket approval of Epi proColon test. http://www.accessdata.fda.gov/cdrh_docs/pdf13/p130001a.pdf
2. Weiss, G. et al., Validation of the SHOX2/PTGER4 DNA Methylation Marker Panel for Plasma-Based Discrimination between Patients with Malignant and Nonmalignant Lung Disease. Journal of Thoracic Oncology, in press. doi: http://dx.doi.org/10.1016/j.jtho.2016.08.123

TT18. Mass Spectrometric Imaging for the Molecular Analysis of FFPE Tumor Sections

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Introduction: Mass spectrometric imaging is an upcoming technique for the investigation of tissues. It is a label-free technique that can directly measure molecular distributions in their histological context. For each pixel, a complete mass spectrometric profile is measured. These molecular phenotypes can be used for spatial segmentation or for classification of tissue samples. This offers the possibility to assess phenotypic heterogeneity in areas that appear histologically homogenous. Recent improvements in sample preparation and instrumentation have led to increased data quality. Unlike other multivariate molecular techniques, MALDI imaging maintains spatial integrity and allows a detailed comparison with histology. Here, we show a detailed analysis of 2 sections, a pancreatic tumor and a lung tumor, with a focus on spatial segmentation based on mass spectrometric molecular phenotypes. **Methods:** Formalin-fixed paraffin-embedded (FFPE) pancreatic tumor tissue was cut at 5 µm and subjected to deparaffinization and heat-induced epitope retrieval. Trypsin solution (0.1 µg/µl) was sprayed on the tissue slice using an automatic spray instrument. After 1.5 h digestion, alpha-cyano-4-hydroxycinnamic acid (CHCA) matrix was deposited using the same spray device and the tissue was analyzed for peptides with a matrix assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometer (rapifleX MALDI Tissuetyper, Bruker). Ion images and digital slides of the H&E stains were visualized using flexImaging software at 50 µm spatial resolution. Mass spectra were imported into SCiLS Lab software (SCiLS GmbH) for segmentation feature extraction and statistical analysis. **Results:** The objective of this study was to show detailed different histological structures by looking at the untargeted protein expression using a pancreatic cancer and a lung cancer section as examples. The entire pancreatic cancer section was subjected to hierarchical cluster analysis that allowed statistical grouping of similar spectra. Pixels belonging to a particular cluster were then assigned to a selected color and displayed as a spatial segmentation map. The clusters correlated with 8 different histological areas present in the tissue. In addition, for each cluster individual molecule species were found to be highly correlated to a distinct histopathological entity. Similar results were obtained for the lung section. **Conclusions:** The presented results show that it is feasible to obtain high-quality images and molecular data using mass spectrometry. Using this data, it is then possible to classify tissue sections according to their molecular profiles.

TT19. Application of the GeneReader NGS System in Clinical Testing of Tumor Samples

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Introduction: At Weill Cornell Medicine, we have implemented Next Generation Sequencing (NGS) for the identification of genetic aberrations in tumors for the past 2 years. The QIAGEN GeneReader NGS System is a fully integrated system aimed at helping community hospitals to adopt this technology. To this end, we tested its performance and compared against our existing workflow using the Ion Torrent Personal Genome Machine (PGM). **Methods:** We tested tumor FFPE sections from various tissue sources including Colon, Lung and Pancreas with previously known mutational status. Fifty-nine samples carrying 1 or more clinical mutations between 5% and 88% Variant Allele Frequency (VAF) were processed using the GeneReader NGS System. DNA was extracted with the QIAGEN DNA FFPE Kit, library prepared and sequenced using the GeneReader, and results analyzed using the QIAGEN Clinical Insight (QCI) Analyze (for secondary analysis) and QCI Interpret (for variant interpretation). Samples with variants in *BRAF, KRAS* and *NRAS* genes

were run in triplicates to test for precision and reproducibility at the Single Nucleotide Variant (SNV) level, in accordance with New York State Department of Health (DoH) NGS Guidelines for Somatic Variant Detection (published in March 2016). These data were then confirmed with previous results from Ion Torrent PGM. **Results:** Samples were run in a 10-plex configuration per flowcell according to the manufacturer instructions, and yielded sequencing results with an average coverage of 5920 at hotspot level. All variants in 52 clinical samples were confirmed. The allele frequency comparison with PGM results showed more than 98% and 96% agreement with regard to *KRAS* and *NRAS* variants, respectively. *BRAF* variants reported, had >96% agreement but for 1 sample where GeneReader showed half the allele frequency expected with PGM. On the other end direct VAF comparison for *EGFR* showed less agreement (about 75%) between platforms due to differences when comparing allele frequencies for the larger deletion (ΔE746-A750) in exon 19 of the *EGFR* gene. This discrepancy in allele frequencies might be due to the respective panel design developed for the different platforms and the challenge to resolve large deletions. Additionally, all triplicate samples with SNVs run on the GeneReader system demonstrated 100% confirmation of the variants, with excellent concordance even at low VAF. **Conclusions:** The QIAGEN GeneReader System is a reliable NGS platform that yields accurate and consistent results for SNVs, at a standard that meets the NY State DoH Guideline requirements. With its fully integrated sample preparation, quality checks and bioinformatics solutions, it is well positioned for clinical laboratories desiring to implement the NGS technology routine analysis.

TT20. Analysis of Clinical Relevant Mutations by the Novel Gene Reader NGS System

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Introduction: For oncology diagnostic applications in which tumor biopsy tissue is often limited, the ability to gain multiple insights from minimal sample material is particularly beneficial. Targeted application of NGS technology can enable the identification specific alterations that affect the evolution of the disease and its response to a particular regiment, thus providing an essential tool in guiding clinical decisions. In the present study, we applied the novel Gene Reader NGS system to analyze clinically relevant mutations in various cancer types. **Methods:** Hereby, we conducted an extensive performance comparison between the GeneReader NGS System workflow with an alternative NGS platform. We tested 49 formalin fixed and paraffin embedded (FFPE) clinical tumor samples taken from a range of cancer types (colorectal carcinoma, lung, melanoma, GIST and stomach) with the GeneRead QIAact Actionable Insights Tumor Panel. Mutational status and variant allele frequencies were compared, and verified via Sanger sequencing. In addition, we assessed the system's usability in a clinical pathology laboratory setting. **Results:** The Actionable Insights Tumor Panel investigates 773 variant positions in 12 genes previously shown to be of high clinical relevance. 40 samples form the total of 49 samples showed a region of interest (ROI) coverage higher than 200x and 34 out of 50 samples met the 500x coverage criteria. In total, there were 56 mutations with clinical relevance found. The same DNA extracts, used for the GeneReader NGS study were previously used for a MiSeq workflow or the Sanger sequencing technology. Notably, we demonstrate a 100% agreement between GeneReader NGS system and the previously used MiSeq pipeline. **Conclusions:** The GeneReader NGS System provides a full sample-to-insight solution. The system allows a seamless workflow to be easily integrated into any laboratory. Targeted sequencing of only clinically actionable 'hotspots' produces 'necessary and sufficient' set of data with maximal testing efficiency and minimal unnecessary information.

TT21. Novel rhPCR Assays for Sensitive and Accurate Detection of Germline SNPs and Somatic Mutations

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Introduction: We report here a novel universal probe-based rhPCR genotyping assays for specific and sensitive detection of both germline and somatic DNA variations. This technology utilizes a Type II RNase H (RNase H2) in conjunction with a mutant Taq polymerase with high allele discrimination to increase the specificity, selectivity and multiplexity of the PCR. A universal reporter system was utilized to achieve a cost-effective genotyping solution and enables multiple fluorophore detection of multi-allelic variants in a single reaction. This approach is particularly useful in pharmacogenetics where increasing number of tri- and tetra-allelic SNPs are found to have pharmaceutical and clinical value. High specificity and sensitivity of multiplex rhPCR assays are also suitable for detection of somatic mutations in cancer. **Methods:** In this study, we designed a collection of > 150 assays detecting potentially causative SNP, MNP or InDel polymorphisms in DME genes including tri-allelic targets. The assays were designed using an optimized assay design algorithm ensuring high target specificity. All assays were tested with 137 Coriell DNA samples from 3 populations and synthetic templates representing each genotype. Two multiplex rhPCR assays targeting four RDH1 (c.395G>A, c.394C>T, c.394C>A, and c.394C>G) and two RDH2 (c.419G>A and c.515G>A) mutations, respectively were designed and tested with synthetic gBlock gene fragments with mutant alleles which were spiked into normal Coriell gDNA samples to measure the assay sensitivity and

specificity. **Results:** We successfully demonstrated >95% coverage for the desired targets (including multi-allelic SNPs), and >99% call rate and 100% accuracy were achieved for DME SNPs in the tested samples. Genotyping results are concordant with capillary electrophoresis sequencing data. By combining both real-time and end-point rhPCR assays, allelic copy number variation of DME SNPs in genomic DNA samples could be successfully determined. For multiplex IDH1/2 assays, there were >10 Cq differences between matched and mismatched loci at 1,000 copy DNA input, suggesting its detection sensitivity at 1% or better. **Conclusions:** Results suggest that rhPCR genotyping technology provides a highly specific genotyping solution at a lower cost. Multiplex detection with multiple universal probes allows us to perform any multi-allelic or multi-loci SNP genotyping in a single reaction.

TT22. Development of a Breast and Lung Cancer Research Panel To Target Therapeutically Relevant Copy Number and Gene Fusion Variants from Blood

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Introduction: With recent advances in next-generation sequencing (NGS) technologies, it is now possible to detect somatic mutations with allele frequencies in blood samples as low as 0.1% from circulating tumor DNA. A natural extension to this achievement is adding the ability to simultaneously detect copy number variants and gene fusions. A panel such as this addresses a full repertoire of variant classes found to be linked with certain tumors and would enable researchers additional to profile cancer samples more dynamically thus enriching current diagnostic tool sets. Here, we present progress on such an approach and apply current NGS technology to achieve our goals. **Methods:** Using control samples, we can reproducibly demonstrate detection of ERBB2 (HER2/neu) gene amplifications with high statistical significance and as low as a 2 fold difference versus non-amplified loci in titration experiments. In addition, this ERBB2 gene amplification was detected in the context of a validated breast cancer somatic mutation panel in which no negative impact was exhibited and mutation detection specificity and sensitivity were both greater 90%. Lastly, we developed an additional panel to detect gene fusions relevant to lung cancer. **Results:** Using the titration approach above, the EML4-ALK fusion variant was shown to have a limit of detection near 1% with no negative impact on detection sensitivity and sensitivity when combined with the validated lung cfDNA somatic mutation panel. **Conclusions:** From the outcomes of these experiments, we have shown the ability to reproducibly and simultaneously detect copy number and gene fusion variants as well as somatic mutations at very low limits of detection in a cell free DNA background derived from blood samples.