#536 Poster Session (Board #28), Sat, 8:00 AM-11:30 AM

Breast cancer cell-free DNA (cfDNA) profiles reflect underlying tumor biology: The Circulating Cell-Free Genome Atlas (CCGA) study.

First Author: Minetta C. Liu, Mayo Clinic, Rochester, MN

Background: New breast cancer screening approaches are needed to detect clinically aggressive subtypes that may not be detected by mammography or are detected late in unscreened populations. CCGA (NCT02889978) is a prospective multi-center observational study for the development of a noninvasive assay for cancer detection. A preplanned substudy of a Women- Only Cohort is reported. Methods: Blood was prospectively collected (N = 1627) from 878 participants (pts) with newly diagnosed untreated cancer (20 tumor types, all stages) and 749 pts with no cancer diagnosis (controls, C) for plasma cfDNA extraction. This substudy included 358 pts with in- vasive breast cancer (IBC) and 452 C. Three prototype sequencing assays were performed: paired cfDNA and white blood cell (WBC) targeted se- quencing (507 genes, 60,000X) for single nucleotide variants/indels, paired cfDNA and WBC whole genome sequencing (WGS, 30X) for copy number variation, and cfDNA whole genome bisulfite sequencing (WGBS, 30X) for methylation; WBC sequencing identified the contribution of clonal hema- topoiesis (CH). For each assay, a classification model using 10-fold cross- validation was developed to discriminate IBC from C using a subset of women; sensitivity was estimated at 95% specificity. Results: IBC pts and C had similar age (mean yrs6SD: 58613 IBC, 59612 C). 46% of IBC pts were symptomatic (a subset were documented interval cancers), 82% were stage I/II. The subtype breakdown of HR+/HER2+/triple-negative breast cancer (TNBC) was 65%/17%/15%. WGBS returned the highest sensitivity of the 3 assays and is reported here; results were consistent across all assays. Sensitivity (95% CI) was higher for TNBC vs HER2+ vs HR+/HER2- (58% [43- 72] vs 40% [28-54] vs 15% [10-20]), and higher for symptomatic vs screen- detected breast cancer (44% [36-52] vs 10% [6-16]). Comparison to tumor WGS and multi-assay classification will be reported. Conclusions: Breast cancers with detectable cfDNA signals at time of diagnosis included clinically aggressive subtypes and symptomatic presentation. Further assay and clinical development in the intended use population is ongoing (NCT03085888). Clinical trial information: NCT02889978.

#12003 Oral Abstract Session, Tue, 8:00 AM-11:00 AM

Prevalence of clonal hematopoiesis of indeterminate potential (CHIP) measured by an ultra-sensitive sequencing assay: Exploratory analysis of the Circulating Cancer Genome Atlas (CCGA) study.

First Author: Charles Swanton, Translation Cancer Therapeutics Laboratory, The Francis Crick Institute, London, United Kingdom

Background: CHIP is defined by the presence of age-dependent acquired mutations in hematopoietic progenitor cells and has been reported to occur in up to 30% of individuals 60-70 years of age. CHIP is a risk factor for hematologic malignancies and cardiovascular disease; its biological mecha- nisms and clinical significance are just now being studied. Using an assay ~100X more sensitive than exome sequencing, we determined the prevalence and features of CHIP in the CCGA cohort, and the impact on interpretation of cell-free DNA (cfDNA) somatic variants. Methods: Blood was prospectively collected (N = 1627) from 749 controls (no cancer, C) and 878 participants (pts) with newly-diagnosed untreated cancer (20 tumor types, all stages) for WBC and cfDNA isolation. Paired white blood cell (WBC) and cfDNA targeted sequencing (507 genes, 60,000X median coverage) identified somatic single nucleotide variants/indels. Unique molecular barcodes and a machine learning-based noise model achieved a specificity of 1 false positive variant call per Mb of genome targeted at a limit of detection of ~0.1% variant allele frequency (VAF). Results: 1412 samples were eligible and evaluable (576 C, 836 pts; 18 solid tumor types, all stages). Of somatic cfDNA variants matched in WBC (CHIP), 7% of individuals had CHIP with VAF . 10%, 39% had CHIP with VAF . 1%, and nearly all pts (92%) had a somatic mutation with VAF . 0.1%. The rate was similar between C and pts (median age 62, 60), increasing in prevalence by 160% per decade, such that we observed 2.5 variants/Mb at age 60. Of CHIP variants identified, 92% were unique to individual patients, most of which were present at low VAF. Genes impacted by CHIP included DNMT3A (40%), TET2 (27%), and TP53 (10%), consistent with previous reports in patients with solid tumors. Conclusions: An ultra- sensitive sequencing assay demonstrated that CHIP signal in WBC and cfDNA is much more common than previously appreciated. The clinical significance of CHIP warrants further study and must be accounted for when interpreting cfDNA variants for both early cancer detection and tumor genotyping (liquid biopsy). Clinical trial information: NCT02889978.

#12021 Poster Discussion Session; Displayed in Poster Session (Board #134), Mon, 1:15 PM-4:45 PM, Discussed in Poster Discussion Session, Mon, 4:45 PM-6:00 PM

Development of a comprehensive cell-free DNA (cfDNA) assay for early detection of multiple tumor types: The Circulating Cell-free Genome Atlas (CCGA) study.

First Author: Eric A. Klein, Cleveland Clinic Glickman Urology and Kidney Institute, Cleveland, OH

Background: Globally most cancers are detected at advanced stages with high treatment burden and low cure rates. A noninvasive cfDNA blood test detecting multiple cancers at early stages when curative treatment is more likely to succeed is desirable. CCGA (NCT02889978) is a prospective multi- center observational study for development of a noninvasive cfDNA-based multi-cancer detection assay. Methods: Prospectively collected samples (N = 1627) from 749 controls (no cancer diagnosis, C) and 878 participants (pts) with newly diagnosed untreated cancer (20 tumor types, all stages) were analyzed in a preplanned substudy. 3 prototype sequencing assays were performed: paired cfDNA and white blood cell (WBC, 60,000X) targeted sequencing (507 genes) for single nucleotide variants/indels; paired cfDNA and WBC whole genome sequencing (WGS, 30X) for copy number variation; cfDNA whole genome bisulfite sequencing (WGBS, 30X) for methylation. For each assay a detection model was developed for all cancer pts; sensitivity was estimated at 95% specificity. Results: Pts w/cancer and C had similar age, smoking status and gender. WGBS had the highest sensitivity and is reported here; results were consistent across assays. Detected (sensitivity [95% CI]) cancers (stage I-III) included 28 colorectal (66% [48-84]), 19 esophageal (63% [38-84]), 5 head and neck (56% [21-86]), 5 hepatobiliary (80% [28-99]), 73 lung (59% [47-70]), 17 lymphoma (77% [50-93]), 11 multiple myeloma (73% [39-94]), 10 ovarian (90% [56-99]), and 10 pancreatic (80% [44-98]). Breast cancer-specific assay results are reported separately. Cancers with low signal (, 10% sensitivity) include low gleason score prostate cancer, thyroid, uterine, melanoma, and renal. Comparison to tumor WGS and multi-assay classification will be reported. Conclusions: A cfDNA-based blood test detected multiple cancers at various stages with high specificity, indicating this approach is promising as a multi-cancer screening test, including for lethal unscreened cancers where stage shift can impact mortality. Further assay and clinical development of a multi-cancer cfDNA test in an asymptomatic population is ongoing. Clinical trial in- formation: NCT02889978.