immunotherapies targeting programmed death-1 (PD-1)/PD ligand 1 (PD-L1) axis yield promising results. The current data are insufficient to characterize the molecular alterations in PPC. **Methods:** Our pathology and clinical information systems were searched for PPC that underwent resection between 2011 and 2023. Pathology, demographics, clinical, and tumor molecular profiles were reviewed. **Results:** Seventy-three cases of PPC were identified across 7 hospitals. Forty-eight (66%) were men (mean age 71.7y), and 25 (34%) were women (mean age 67.7y). Racial distribution was: 43 White, 4 Black/African American, 18 Asian, 7 Other, and 1 American Indian or Alaska native. Fifty-five (75%) had a history of smoking, 9 (12.3%) had never smoked, and 9 were unknowns. Seventy were unifocal, and 3 multifocal (all right lung), 46 (63%) in the right lung (RUL: 32, RML: 5, RLL: 11), and 24 (33%) in the left lung (LUL: 12, LLL: 10, Lingula: 2). Seven cases had chest wall invasion, and 3 had mediastinal invasion. Twenty-three (31%) cases had metastasis (10 brain, 8 bone, and 5 adrenal gland). Epithelial component types were: 38 adenocarcinoma, 16 squamous cell carcinoma, and 4 large cell carcinomas. T staging: T4: 6, T3: 12, T2b: 6, T2a: 26, T1c: 3, T1b: 8, T1a: 6, and Tx: 6. A total of 68.5% of cases were resected at stage ≥T2a. N staging was N2: 8, N1: 15, N0: 42, and Nx: 8. Programmed cell death 1 (PD-L1) was measured in 35 cases, and 29 (83%) were positive (24 with score >50). Molecular alterations (percent alteration/tested): *TP53* 85%/27, *KRAS* 75%/24, *TERT* 57%/7, *STK11* 57%/7, *PIK3CA* 20%/10, *EGFR* 17%/30, *BRAF* 15%/20, *NRAS* 12%/8, *MAP2K1* 11%/9, and *MET* 6%/18. No alteration was found in *AKT1, ALK, ATR, CHEK1, CCND1, DDR2, ERBB2, ERBB3, FGFR1, NTRK, POLD1, POLE, RET*, and *ROS1*. There were 3 alterations in *DNMT3A*, 3 in *NF1*, and 1 in *HRAS, SMARCA4*, and *CDKN2A/B*. Six-month, 1-year, and 5-year survival in patients with available survival data were 90.2%, 76.8%, and 37.4%, respectively. In patients with survival <1 year, co-mutation of *KRAS* and *TP53* were identified (PD-L1 score >50, 85%). **Conclusions:** PPC is more prevalent in elderly men with a smoking history. *TP53* and *KRAS* are the most common genetic mutations, followed by *TERT, STK11, PIK3CA, EGFR*, and *BRAF*. Higher TNM stage, metastasis, and co-mutation of *KRAS* and *TP53* are associated with worse prognosis, and aggressive treatment should be considered. PPC has a high PD-L1 score rate, which makes immunotherapy a promising treatment. Identification of additional actionable mutations will improve treatment and overall survival of PPC.

ST-13

Comprehensive Genomic Profiling of Solid Tumor Patients with the OncoDEEP Assay for Broad Analysis in Clinical Diagnostics

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Introduction: Somatic multigene analysis by next-generation sequencing (NGS) becomes standardly integrated in medical oncology for clinical decision making. However, with the fast-growing number of recommended and required genomic biomarkers, small panels have become vastly insufficient for most tumor types. Comprehensive genomic profiling (CGP) is amenable to screen for single nucleotide variants (SNVs) and indels in several hundred genes. Moreover, it provides information on amplifications and gene fusions of the most relevant genes for optimal patient management. Currently, most comprehensive panels also allow for screening of tumor-agnostic genomic biomarkers including microsatellite instability (MSI), tumor mutational burden (TMB), and homologous recombination deficiency (HRD), which are implemented as prognostic and therapeutic signatures in a wide variety of solid tumors. So far, only a handful of CGP assays have been validated for their diagnostic utility in routine laboratory practice for the care of cancer patients. **Methods:** We report on an extensive multicentric analysis across 7 NGS centra in Belgium and the Netherlands comparing the novel OncoDEEP CGP assay (OncoDNA) with the diagnostically validated TSO500 assay (Illumina). We describe the technical differences between both assays as well as their outcome and shortcomings based on the comparative analysis of 160 retrospective diagnostic formalin-fixed, paraffin-embedded samples. For the diagnostic implementation of the OncoDEEP assay we performed an extensive validation with clinical samples representing a wide variety of solid tumor types, as well as on reference samples. **Results:** Detection of clinically relevant SNVs, indels, and copy number variants was highly concordant, with both

assays yielding very similar variant allele frequencies (VAF) or fold changes (FC). Most differences were due to assay-specific settings such as VAF and FC thresholds. Similarly, TMB, MSI, and HRD data were concordant for most samples, although those with scores close to the cut-offs could deviate qualitatively. For fusion detection, a significant higher starting amount of RNA (200 ng) is required for the OncoDEEP analysis compared to TSO500 (40 ng), but concordance was found for the limited number of 11 clinically actionable driver genes covered by the OncoDEEP kit. The uniformity of coverage, however, was higher with the OncoDEEP assay, thereby allowing pooling of at least 2 times more samples per NGS run. In the validation experiments, all performance metrics passed the validation criteria. Finally, the OncoKDM pipeline provides fully automated variant interpretation and reporting, whereas currently this has to be done manually for the TSO500 pipeline. **Conclusions:** The OncoDEEP CGP assay provides highly similar data as compared to the validated TSO500 assay but includes a workflow from library preparation to report. Validation demonstrated it can reliably be used for diagnostic profiling of solid tumors, but currently extensive fusion analysis requires an additional screening method.

ST-14

Detection of Common Hotspot Variants in *PIK3CA* and *TP53* Using Agena ClearSEEK on the MassARRAY System

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Introduction: The *TP53* and *PIK3CA* oncogenes are the most frequently mutated genes across all cancer types and in breast cancer, respectively. Mutational hotspots are a recurrent feature in both genes which, due to positive selection during tumorigenesis, can be potentially exploited by targeted treatments, as has been demonstrated by the FDA-approved PI3K inhibitor alpelisib in advanced hormone-receptor positive (HR+) breast cancer. The Agena ClearSEEK PIK3CA Panel covers all major hotspot mutations (N = 20) with evidence level 1 for the prediction of alpelisib benefit. The Agena ClearSEEK TP53 Panel is designed to test for hotspot mutations including the 11 most common missense/nonsense substitutions, comprising »30% of all *TP53* mutations. **Methods:** Mutational profiling was performed using the Agena ClearSEEK PIK3CA Panel v1 (N = 20 variants) and Agena ClearSEEK TP53 Panel v1 (N = 17 variants) in 345 and 634 formalin-fixed, paraffin-embedded (FFPE) tumor specimens, respectively, from a single HR+ breast cancer cohort. A total of 20ng FFPE-derived tumor-enriched DNA was subjected to a single global PCR reaction, and subsequently divided into 3 (*PIK3CA*) or 4 (*TP53*) multiplexed extension reactions. Purified reactions were analyzed with the MassARRAY System (Agena Bioscience). Sensitivity and specificity were tested by a platform comparison to digital droplet PCR (ddPCR, Bio-Rad GmbH). **Results:** ***PIK3CA*:** Mutation calling was successful in 337 (98%) specimens. The number of mutated tumors was 152 (45%), including 11 (3.2%) and 3 (0.9%) tumors with double- and triple-mutations, respectively. Fourteen different *PIK3CA* mutations were detected at variant allele frequency (VAF) between 0.5% and >10%. The previously observed concordance rates with ddPCR for the most abundant and clinically relevant variants E542K, E545K, and H1047R were confirmed, i.e., 97% to 98% in the low performance range and 100% in unambiguous variant calls (>20 droplets or >1% VAF). ***TP53*:** Following rigorous validation using ddPCR for most of the *TP53* panel variants, the mutation status of 608 (95%) tumor specimens was successfully assigned. Of the 22 tumors (3.6%) that were called *TP53*-mutated, R273H, R248Q, R175H, and Y220C were most common (range N = 3 to 5 patients per single variant). A test of correlation between detected variants and an mRNA-derived p53 signature score, previously trained by functional and mutation associated gene expression data, revealed significantly higher p53 scores (P = 9.9e-05), indicating the biological relevance of the *TP53* panel variants. **Conclusions:** The Agena ClearSEEK PIK3CA and TP53 Panels combine low hands-on time requirements with accurate data assessment and provide a reliable tool for clinical trial evaluation of known actionable *PIK3CA* mutations and response to PI3K inhibitors in breast cancer, as well as for investigating the oncogenic activities of *TP53* hotspot mutations and patient selection, e.g., for cell cycle targeting therapies.

ST-15

Findings of Molecular Alterations Determined by Next-Generation Sequencing Target in Mexican Patients with Gastrointestinal Carcinomas Using a Multi-Tumor Panel

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Introduction: Gastrointestinal (GI) cancer is a malignancy of the gastrointestinal tract with high global morbidity and mortality. Survival of patients with gastrointestinal cancer tends to be low, and it is a highly heterogeneous disease distinguished by multiple genetic and epigenetic events critical for tumor initiation and progression.