the QIAsymphony® SP instrument using the QIAsymphony PAXgene Blood ccfDNA Kit* and protocol. ccfDNA stability after blood storage was confirmed by qPCR. ccfDNA samples were sequenced on the QIAGEN GeneReader® NGS System, including the GeneRead QIAact Actionable Insights Tumor (AIT) Panel* for PCR target enrichment, library preparation on the QIAcube® instrument, QC with capillary electrophoresis, sequencing on the GeneReader instrument* and data management with the QIAGEN Clinical Insight (QCI) Analyze* tool.

Results After blood storage for 7 days, ccfDNA yield was similar to ccfDNA yield observed directly after blood draw. All samples (60/60) passed the required QC criteria after target enrichment (amplicon size around 160 bp) and library preparation (amplicon size around 252 bp and absense of unspecific products < 170 bp). Also, all GeneReader NGS acceptance criteria that defined passed or out-of-spec samples were passed after sequencing, including reads above average quality 25 with $90.16 \pm 2.04\%$ (acceptance criteria $> 80\%$), region of interest with coverage of bases $> 500\times$ with $99.83 \pm 0.47\%$ ($> 90\%$) and region of interest with coverage of bases $> 200\times$ with $99.99 \pm 0.03\%$ ($> 95\%$). Across all samples, 17 ± 5 different variants in 7 ± 1 different genes were identified with the AIT targeted panel.

Conclusion NGS is one of the most important applications for analysis of ccfDNA in research and clinical settings. This study verified that ccfDNA stabilized and extracted with the PAXgene Blood ccfDNA System is highly suitable for NGS applications, meeting quality control acceptance criteria for 100% of analyzed samples. *For Research Use Only. Not for use in diagnostic procedures.

P-35 Detection of tumor-associated gene mutations in cell-free DNA of early-stage non-small cell lung cancer patients

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Introduction Recently, the gene analysis using circulating cell-free DNA (cfDNA) gives us an increasing interest as a tool of non-invasive biomarker for treatment selection in advanced non-small cell lung cancer (NSCLC). However, few studies have reported in early-stage NSCLC. The aims of this study are to elucidate the sensitivity of tumor-associated gene mutations in cell-free DNA and the correlation between the sensitivity and patients' characteristics.

Material and methods From January 2017 to June 2017, patients with suspected NSCLC who underwent curative resection in our hospital were enrolled in this study. This study was approved by the Institutional Review Board of Kanazawa University Hospital. Written informed consent was obtained from all patients. Tumor DNA was extracted from 25 mg of tumor tissues using QIAamp DNA Mini Kit (QIAGEN). Blood samples were collected within 14 days of resections, and cfDNA were extracted immediately using QIAamp Circulating Nucleic Acid Kit (QIAGEN). EGFR and KRAS mutation were analyzed by droplet digital PCR (Prime PCR, Bio- RAD). Concentrations of cfDNA were calculated by real-time quantitative PCR targeted ALU gene.

Results 40 patients were enrolled, and the mutations were detected in 14 (35.0%). All patients with the mutations had adenocarcinoma histology. Deletional mutation (DEL) and L858R in EGFR, and KRAS mutation were detected in 5, 6 and 4 respectively. The sensitivity of mutations for paired tumor DNA and cfDNA was 33% (5/14). All patients with mutations in cfDNA had DEL. The concentrations in patients with cfDNA mutations were significantly higher than that without cfDNA mutations (35.4 vs 13.0 ng/mL).

Conclusion The study demonstrate that tumor-associated mutations were detected from about a quarter of early-stage NSCLC patients using droplet digital PCR as a high sensitive assay. Deletinal mutation of EGFR and high concentrations of cfDNA were factors for which tumor-associated mutations is likely to be detected in cfDNA.

P-36 Evaluation of complete and quantitative size distribution of cfDNA as a monitoring marker in oncology

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Introduction cfDNA size in the context of cancer is a subject of intense debates. We have used the BIABooster system from Picometrics Technologies to investigate systematically the size profile of $\sim$ 200 cfDNA samples from patients with colon, gastric, lung, pancreas, and ovarian cancers, as well as from healthy donors.

Material and methods The technology is operated automatically on a commercial capillary electrophoresis instrument using electro-hydrodynamic actuation.

Results Its sensitivity, down to 10 fg/μL, enables the quantification of the whole size distribution of cfDNA for any sample. The total concentration quantification is in good agreement with results obtained by fluorimetry and droplet-based digital PCR. We will show significant differences between patients and healthy donors for almost all the investigated cancers. Moreover, a recent technological development allows to determine the size profile of cfDNA directly from plasma, without prior DNA purification.

Conclusion These features make cfDNA size profiling a good candidate as a monitoring marker in oncology.

P-37 Deep learning to identify circulating tumor cells by ACCEPT

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Introduction The number of circulating tumor cells (CTCs) in blood of patients with metastatic cancer can be used to monitor their treatment. Correct assessment of CTCs is of great importance to accurately monitor the number of CTC during the course of therapy. Consistent manual assessment of objects as CTC can however be very challenging and results are subjective and associated with high inter-reader differences. To arrive at a unified and standardized definition to classify cells as CTCs the open-source toolbox ACCEPT (Automated CTC Classification, Enumeration and Phenotyping) was developed within the EU IMI consortium CANCER-ID. The first version of ACCEPT making use of advanced segmentation algorithms to identify and manually classify objects is freely available at: http://www.github.com/LeonieZ/ACCEPT. Here we compare manually scored CTCs to CTCs identified by advanced deep learning techniques.

Material and methods To train an algorithm to automatically classify objects as CTCs, tumor-derived extracellular vesicles (tdEVs), white blood cells (WBCs) or cells of unknown origin stored images of blood samples processed with the CellSearch system were analyzed using ACCEPT. Samples from 200 breast, 203 colon, 41 lung and 39 prostate samples and 8 samples from healthy controls spiked with cells from tumor cell lines were used for this purpose. The gating function of ACCEPT was used to extract 13.123 CTC, 10.221 WBC, 8548 tdEV and 8599 nucleated cells of unknown origin. These candidates were manually reviewed by several trained reviewers and placed in each category. Based on this ground-truth a deep learning

network was trained on 80% of this data to automatically classify cells into the aforementioned categories. The other 20% of the data were used as a validation set.

Results The network we trained to classify CTCs vs all other objects received an accuracy of 98.62% with a sensitivity of 97.21% and a specificity of 98.97%. Objects wrongly classified as CTC are CK+, DAPI+ and CD45- objects with a doubtful morphology. Misclassified CTCs mainly lack a sufficiently strong CK signal. When concentrating on the more difficult part of classifying CK+, DAPI+ and CD45− into CTCs or no CTCs we receive an accuracy of 93.69% with a sensitivity of 96.92% and a specificity of 77.72%. For some cells misclassified by the algorithm we think, that the algorithm is actually correct and propose that an expert panel reviews the scores of these cells.

Conclusion The use of automated image analysis and classification techniques is necessary to obtain objective CTC counts. This problem is addressed by the development of the ACCEPT open-source toolbox for CTC analysis.

P-38 Dynamics of circulating tumor cells during the course of chemotherapy and prognostic relevance across molecular subtypes in high-risk early breast cancer patients: results from the adjuvant SUCCESS A trial

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Introduction The presence of circulating tumor cells (CTCs) before chemotherapy is associated with reduced disease free survival (DFS) and overall survival (OS) in early breast cancer (EBC). In addition, recent data suggest that CTCs persisting after adjuvant chemotherapy indicate poor prognosis. Here, we evaluate the prognostic relevance of changes in CTC counts during the course of adjuvant chemotherapy across molecular subtypes to assess whether the prognostic role of persisting CTCs varies according to tumor biology.

Material and methods The German SUCCESS A trial is a phase III study, in which patients with high-risk EBC were randomized to adjuvant chemotherapy with 3 cycles of epirubicin-fluorouracil-cyclophosphamide followed by either 3 cycles of docetaxel or 3 cycles of gemcitabine-docetaxel. Presence of CTCs was assessed before and after chemotherapy using the FDA-approved CellSearch® System (Janssen Diagnostics, LLC). CTC positivity was defined as ≥ 1 CTC in 7.5 ml blood, and patients were grouped according to their CTC status before and after chemotherapy as CTC neg/neg, CTC neg/pos, CTC pos/neg, or CTC pos/pos. Molecular subtypes were defined as luminal A like (hormone-receptor positive, grading 1 or 2), luminal B like (hormone-receptor positive, grading 3), triple-negative or HER2-positive. Patient outcome in terms of DFS and OS was analyzed using univariable log-rank tests and Cox regression models (median follow-up time 65.2 months).

Results Data on both molecular subtype and CTC status before and after chemotherapy were available for 1485 (39.6%) of 3754 patients randomized for the SUCCESS A trial. Overall, 917 (61.8%) patients were CTC neg/neg, 260 (17.5%) were CTC neg/pos, 229 (15.4%) were CTC pos/neg, and 79 (5.3%) were CTC pos/pos. There were significant differences in DFS and OS among these four groups in patients with luminal A like tumors (log rank test, both $p < 0.003$) and patients with luminal B like tumors (log rank test, both $p < 0.001$). In both patients with luminal A like and luminal B like tumors, CTC pos/pos patients had the worst outcome (relative to CTC neg/neg patients) in terms of DFS and OS. In contrast, no significant differences with regard to DFS or OS were found among the four groups (neg/neg, neg/pos, pos/neg, pos/pos) in patients with HER2-positive or triplenegative tumors (log rank test, all $p > 0.13$).

Conclusion The presence of CTCs both before and after adjuvant chemotherapy was associated with poor survival in luminal A like and luminal B like tumors, but not in HER2-positive or triple-negative tumors.

P-39 The tumor-initiating capacity of prostate circulating tumor cells examined through a unique CTC-derived eXplant (CDX) model

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Introduction The rarity of in vivo and in vitro human prostate cancer (PCa) models has hampered progress in understanding disease pathogenesis, metastatic progression and drug resistance mechanisms. Using CTCs from a diagnostic leukapheresis (DLA) product of a patient with advanced PCa, we present the establishment, phenotypical and molecular characterization of a CDX and an in vitro cell-line derived from this CDX.

Material and methods Leukapheresis was performed in seven patients with advanced castration-resistant prostate cancer (CRPC). CTCs from leukapheresis were enriched by RosetteSep and implanted in Nod/Scid-IL2Rγ−/−mice. The CDX tumor was propagated in successive generations of mice. All samples, including eight tumor-biopsies performed at diagnosis 2 years prior DLA, CTCs isolated at the single cell level during DLA, CDX and CDX-derived cell-line were characterized by immunofluorescence, immunohistochemistry, and whole-exome sequencing (WES).

Results Based on CellSearch counts, the estimated median number of engrafted CTCs was 698 (range 10–19,988). A mouse engrafted with 19,988 CTCs developed a tumor within 193 days. The CDX and patient tumor biopsies were positive for EpCAM, CK8/18, weakly positive for neuroendocrine marker synaptophysin and negative for vimentin. While biopsies expressed PSA and the androgen receptor, the CDX was negative for both indicating tumor evolution. In contrast to tumor biopsies, the CDX strongly expressed Ki67 and chromogranin A, evidencing emergence of a proliferating neuroendocrine phenotype. The in vitro cell-line formed microspheres, expressed ALDH and CD133 cancer stem-cell markers, and showed 84% genetic similarity with the CDX. A tremendous mutational diversity was observed in leukapheresis CTCs. Among this genomic diversity of CTCs only 0.2% of the mutations present in CTCs were observed in the CDX and associated with their tumorigenicity. Mutational heterogeneity was also observed in tumor-biopsies but at a much lower level than in CTCs. Only 2.3% of tumor-biopsy mutations were conserved in CTCs, the CDX and the cell-line, representing trunk mutations. These trunk abnormalities, included TP53 and NF1 mutations, and TMPRSS2-ERG rearrangement, support both CTC dissemination and tumorigenicity. Late abnormalities detected only in CTCs, CDX and the cell-line but not in biopsies included loss of PTEN, RB1 and APC and could be implicated in disease progression and drug resistance. Genome doubling events occurred in both CDX and CDX-derived-cell-line.

Conclusion We report the first PCa CDX model, demonstrating the tumorigenicity of CTCs from CRPC. This model represents a unique