intratumoral heterogeneity with a minimal invasive approach and easy access. We aimed at assessing the feasibility and clinical utility of ctDNA genotyping for patients with advanced CCA in a group of referral Oncology centers across Spain.

Methods: We genotyped ctDNA from blood samples from patients with advanced CCA, treated between 2019 and 2021 at 9 Spanish University Hospitals. ctDNA sequencing was performed by an NGS-based comprehensive approach using Guardant360™ (G360).

Results: ctDNA sequencing data were available for 112 patients. The main clinical characteristics were as follow: median age was 62.5 years (range: 28-86), 62 patients (55.3%) were men, 65 patients (58%) had intrahepatic cholangiocarcinoma, 70 patients (62.5%) had stage IV disease at diagnosis and 98 patients (87.5%) had received cisplatin plus gemcitabine as first-line treatment for advanced disease. Determination of ctDNA was performed at baseline in 36.6% of patients and at the time of progression to first-line treatment in 48 patients (42.8%). 96% of patients had $\geq$ 1genomic alteration detected. Median number of alterations per patient was 2 and median VAF was 0.5%. 245 genomic alterations (GAs) have been identified among 35 different genes. 89% of GAs identified were SNVs, being the most frequent TP53 (33.9%), KRAS (9.8%), IDH1 (6.5%), ATM (6.5%), PIK3CA (4.9%) and ARID1A (4.5%). CNVs accounted for 7.7% of GA and consisted in amplifications of: EGFR (1.6%), MYC (1.6%), BRAF (1.2%) and ERBB2 (0.8%). FGFR2 and FGFR3 fusions were identified in 1.2% and 0.4% patients, respectively. High-microsatellite instability was identified in 1.2% of patients. These findings are similar to previously reported data on tissue molecular profiling in CCA. Importantly, sixty-seven patients (53%) had GA considered as actionable, including three patients with FGFR2 fusions, for which Pemigatinib is approved.

Conclusions: ctDNA genotyping for molecular profiling of advanced cholangiocarcinoma patients is feasible and a non-invasive procedure, especially relevant for CCA patients in which increase in actionable biomarkers and insufficient tissue availability is becoming a challenge.

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PD-11 In depth analysis of label-free infrared (IR) imaging-based microsatellite instability (MSI) classification in early colon cancer (CC) on samples from the AIO ColoPredictPlus 2.0 (CPP) registry trial

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Background: MSI occurs in 15-20% of all early CC due to a deficient mismatch repair system and testing is now considered standard for all colon cancers as MSI is of prognostic and predictive value. Presently, protein analysis using immunohistochemistry targeting the mismatch repair proteins is most frequently used in routine clinical pathology with high sensitivity. Alternatively, fragment length analysis of microsatellite loci or next-generation sequencing can be used. All the techniques referred to are time and tissue consuming. We utilize label-free quantum cascade laser (QCL) based IR imaging combined with artificial intelligence (AI) approaches to test unstained tissue samples for MSI. IR imaging is an emerging microscopic technique based on the interaction of electromagnetic waves with the molecules within the tissue creating molecular fingerprints. To establish the methodological robustness of our approach, we analyzed samples from the multicentric prospective AIO ColoPredict Plus (CPP) 2.0 registry trial.

Methods: IR imaging can analyze unstained paraffin-embedded tissue slides within an average of 30 min/slide. All tissue samples were obtained from the CPP registry trial. For development of the MSI/MSS classification model the cohort was split into a training, test, and validation set. The training set was utilized for the optimization of a modified VGG-16 convolutional neural network (CNN) with area under receiver operating characteristic (AUROC) and area under precision recall curve (AUPRC) evaluated on the test set as endpoints. The final classification model is validated on the validation set and in depth analyzed for sub-cohorts (BRAF, KRAS, UICC stage, grading).

Results: A cohort of 547 patients (training n=331 (43% MSI), test n=69 (43% MSI), validation n=147 (18% MSI)) was selected from CPP. Baseline characteristics including BRAF mutations, were balanced between training and test set, thus providing a balanced ground-truth for the training of the classifier. By contrast, the validation set (18% MSI) corresponds to the natural occurrence of MSI, showing a typically pattern for MSI and MSS with a higher proportion of female patients, more right-sided cases and more BRAF mutations for MSI-samples. The selected MSI/MSS classifier reached a validation AUROC of 0.90 (sensitivity 85%, specificity 84%) and AUPRC of 0.74. The AUROC of the sub-cohorts range between 0.78 and 0.99 (BRAF: 0.93 (wt), 0.78 (mt); KRAS: 0.84 (wt), 0.99 (mt); Grading: 0.93 (G2), 0.84 (G3); UICC Staging: 0.93 (II), 0.86 (III)).

Conclusions: AI integrated IR imaging demonstrates a reliable classification performance for MSI/MSS with an AUROC of 0.90 (sensitivity 85%, specificity 84%) and robustness over sub-cohorts e.g., BRAF, KRAS, or staging. It further approaches the performance of the present gold standard immunohistochemistry. The combination of spatial and biochemical information encoded in the IR images allows CNNs to track changes on the molecular level. Additionally, this method requires fewer samples than other AI approaches and maintains intact tissue for further molecular analysis. Overall, this method has the potential to become an applicable diagnostic tool beyond the scope of known biomarkers for tumor-agnostic assessments.

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PD-12 Characterization of best responder patients to oxaliplatin rechallenge in patients with refractory metastatic colorectal cancer (mCRC)

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Background: Oxaliplatin in combination with fluoropyrimidines constitute one of the most effective treatments in front-line mCRC patients. Rechallenge with oxaliplatin-based regimens in the refractory setting is practiced although based on scarce and heterogeneous evidence. Further clinical and molecular data to determine which patients do benefit from this treatment strategy are needed.

Methods: We analyzed all patients treated with oxaliplatin in a third- or fourth-line setting in our institution between 2015 and 2021. Outcomes were analyzed with overall response rate (ORR), disease control rate (DCR) and median progression free survival (mPFS). The best-responders group was defined-as those patients who achieved mPFS $>$ 6 months. As part of the descriptive analysis, we divided patients into three clinical groups according to the prognostic characteristics previously reported: Good Prognostic Characteristics (GPC) defined as having passed $\geq$18 months since metastatic disease debut, $<$ 3 metastatic sites and presence of liver metastasis, Best Prognostic Characteristics (BPC) defined as $\geq$18 months since metastatic disease debut, $<$ 3 metastatic sites and absence of liver metastases and Poor Prognostic Characteristics (PPC) defined as $<$ 18 months since metastatic disease debut and/or with $\geq$3 metastatic sites. An Amplicon-seq panel was used to analyze regions of interest in 61 genes using an Illumina sequencing platform.