Conclusions: The role of PARP1 in chemotherapy resistance is well established and PARP inhibitors have been extensively utilized in clinical trials to improve chemotherapy response. Our findings suggest that the *PARP1* SNP rs1805407 is linked to a potentially more active form of PARP1 that increases resistance to TMZ. This was supported by findings from the NCI-60 cell lines. We are validating those results in appropriate cell line models. The *PIK3R1* SNP highlights the role of altered signaling pathways in melanoma and its functional role is being examined. The *PARP1* SNP rs1805407 may serve as a biomarker for response to TMZ chemotherapy and potentially PARP inhibitor sensitivity in metastatic melanoma.

MC13-0080

Somatic mutations of the EGFR, KRAS and BRAF genes: Heterogeneity in circulating epithelial tumor cells (CETC) as determined using the Cobas® Z 480 analyzer

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Background: Targeted therapies directed specifically against somatic mutations enhancing the activity of signalling pathways have been shown to improve outcome compared with cytotoxic chemotherapies in patients with advanced tumors carrying the respective mutations.

Purpose/Objective: This requires tests to identify such mutations which are mainly performed on formalin fixed material from the primary tumor. However, such material is not always available and, even more importantly, properties of cells with metastatic potential may change during the course of disease. In contrast, using maintrac®, a nondissipative approach avoiding enrichment steps, CETC be detected and individually isolated in almost all patients with lung and colon cancer and melanoma at different times of disease and therefore can provide a liquid biopsy to monitor the course of disease. We, here, report on the successful analysis of such isolated cells for gene mutations in tumor driver genes EGFR, KRAS and BRAF.

Materials and Methods: Blood from patients with non-small cell lung cancer, colon cancer and malignant melanoma was analyzed for cells positive for epithelial antigen (EpCAM) using the maintrac® approach, which avoids cell selection, and an image analysis system or laser scanning cytometry for detection. Between 8 and 20 EpCAM positive cells from each patient were isolated individually using a semiautomated capillary approach and deposited one by one into micro cups. The DNA was subsequently amplified by whole genome amplification and assayed using either the cobas® EGFR Mutation Test, the cobas® KRAS Mutation Test or the cobas® BRAF V600 Mutation Test.

Results: DNA could be amplified from all individually isolated cells. An EGFR mutation was detected in 12% of isolated tumor cells from a patient with non-small cell lung cancer, the KRAS Mutation was detectable in 28% of cells from a patient with colon cancer and the BRAF Mutation in 100% of evaluable cells from a patient with melanoma.

Conclusions: Individually isolating epithelial tumor cells from the peripheral blood from patients with non-small cell lung cancer, colon cancer and melanoma allows not only detect driver mutations in circulating tumor cells but also to determine the frequency of mutated cells. This proves, that at least part of the CETC from the tumor. They can, in the future, be used as markers of response to the action of drugs and contribute insight into how resistance may be acquired.

MC13-0081

Cell cycle regulatory proteins in pancreatic cancer

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Background: Pancreatic ductal carcinoma (PDAC) is one of the most aggressive cancers. Mutations or epigenetic alterations in the tumour suppressor

or cell cycle regulatory genes readjust cellular homeostasis and ability of apoptosis (Lang *et al.*, 1998; Angela *et al.*, 2013). Detection of p53, p21, p27 and cyclin D1 are described with predicting value of radiosensitivity and chemosensitivity (Fu *et al.*, 1998; Wang *et al.*, 2012).

Purpose/Objective: The aim of this study was to determine the affected cell cycle regulatory proteins and correlation of them with Ki-67 in PDAC.

Materials and Methods: Sixty-six consecutive cases of surgically treated PDAC were evaluated retrospectively. The cases were characterised by patient's age, tumour TNM, stage, histological grade (G1-3), resection margins (R0-1). Expression of Ki-67, p53, p21, p27 and cyclin D1 was detected by immunohistochemistry (IHC) in PDAC and benign pancreatic ducts (BPD). Descriptive statistics was performed by SPSS, version 20. The study was approved by Committee of Ethics.

Results: In the whole group, the mean age was 63.5 years [95% confidence interval CI: 60.6–66.0]. The most frequent tumour characteristics were: size >2 cm: 93.9% [83.4–97.8]; T3: 93.9% [85.2–97.5]; N1: 68.8% [56.6–78.8]; stage IIA: 26.6% [17.3–38.5]; stage IIB: 64.1% [51.8–74.7]; G2: 58.5% [46.3–69.7]; R1: 56.5% [44.0–68.1]. The main IHC data are shown in Table 1. Correlation was found between Ki-67 and p21 (p=0.01), p53 and p21 (p=0.01), p53 and p27 (p=0.00), p21 and p27 (p=0.05) expression.

Conclusions: Morphologically, pancreatic cancer is characterized by aggressive growth. The expression of the analysed cell cycle regulatory proteins p53, p21 and cyclin D1 is frequently affected in pancreatic carcinogenesis suggesting possible benefit from targeted treatment. The proliferation activity shows direct correlation with p21, but the mutual association between p53, p27 and p21 can involve more complex mechanisms.

MC13-0082

Salivary gland cancer in two male BRCA gene mutation carriers

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Background: Germ-line mutations in BRCA genes predispose to breast and ovarian cancers in women. In males, BRCA1 and BRCA2 mutations carriers have a lifetime risk of breast cancer of 2% and 7% respectively and they also exhibit an increased risk for prostate cancers. However, involvement of BRCA mutations in other malignancies is still under investigation. No data are available about the correlation between BRCA mutations and increased risk of salivary gland cancers (SGC).

Purpose/Objective: To assess SGC in a cohort of patients undergoing genetic cancer risk assessment.

Materials and Methods: We reviewed all the cases evaluated in our Institution, for whom BRCA mutations assessment was available. Patients with malignant SGC were retrieved.

Results: We evaluated 2260 families registered in our Institutional databases from 1996 to 2012; 637 families were BRCA mutation positive, whereas 1623 index cases tested negative for BRCA mutations. Within mutated families, we identified 801 carriers of BRCA1 mutation, 456 carriers of BRCA2 mutation and 2 carriers with mutations in both genes. The review revealed two males with SGC among the BRCA mutation carriers and no case in the non-mutated group.

A 56 year-old BRCA1 mutation carrier developed an high-grade mucoepidermoid carcinoma of the parotid gland. He was treated with surgery and post-operative radiochemotherapy, and died 4 years later for distant metastases.

A 66-year-old BRCA2 mutation carrier developed a submandibular salivary gland papillary cistadenocarcinoma treated with surgery and radiation. At 68 years, a sovraclavicular node recurrence appeared and was treated with radiotherapy, followed by parapharyngeal relapse with no treatment opportunities. At the same age, he developed a ductal breast cancer.

Abstract MC13-0081 – Table 1. Expression of Ki-67, p53, p21, p27, cyclin D1 in PDAC and BPD

Parameter	Target	Ki-67	p53	p21	p27	Cyclin D1
Positive cases (%)	PDAC	100 [94.3–100]	69.4 [56.9–79.4]	93.6 [84.5–97.4]	100 [94.3–100]	80.9 [69.5–88.7]
	BPD	21.8 [12.9–34.4]	14.0 [7.3–25.4]	37.5 [25.9–50.7]	100 [94.3–100]	18.6 [10.8–30.4]
Range of positive cells (%)	PDAC	1–55	0–97	0–65	6–82	0–65
	BPD	0–4	0–10	0–13	26–90	0–16
Mean count of positive cells (%)	PDAC	20.8 [17.7–24.4]	30.8 [22.8–39.1]	23.4 [18.9–27.9]	34.7 [30.6–39.2]	19.9 [16.1–23.7]
	BPD	0.4 [0.2–0.7]	0.65 [0.2–1.2]	1.5 [0.8–2.4]	51.5 [47.3–55.6]	0.9 [0.3–1.6]