## P1. BIOMARKERS

**P1.01**

PLASMA MICRORNA EXPRESSION ANALYSIS IN DOUBLE-POSITIVE HER2-NEGATIVE BREAST CANCER VERSUS TRIPLE-NEGATIVE BREAST CANCER

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Breast cancer is a complex and heterogeneous disease with respect to histology, cellular origin, genetic alteration, spreading by metastasizing, lack of response to therapy and clinical outcome. More than 1 million women worldwide are diagnosed with breast cancer each year of which 170,000 cases are defined as triple negative breast cancer (TNBC). In the last years, there is an increasing number of studies proposing microRNA (miRNAs) as biomarkers, in several pathologies, including breast cancer. Our study was centered on the assessment of plasma miRNA in double positive (ER+, PR+ and Her2/ neu-) compared with triple-negative (ER-, PR- and Her2/neu-) breast cancer tumors. The study analyzed a total number of 20 breast cancer patients: 7 with double positive Her2/neu negative status and 13 triple-negative cases. Plasma miRNA pattern was explored using PCR-array technology. MiRNAs from plasma caused problems during isolation and analysis; we used cel-miR-39 spike-in control to track recovery rate and for normalization. Total RNA was extracted using the miRNeasy Mini Kit Qiagen. 5 µl of isolated plasma RNA was reverse transcript using miRNA First Strand Kit. Mature miRNA expression was assessed using the Human Breast Panel miScript miRNA PCR Array (MIHS-109Z). The normalization of the experimental data has been done using the ΔΔCt method. miR-141-3p, miR-203a, miR-548c-3p, miR-607 and miR-96-5p were significantly overexpressed, whereas miR-181a was significantly underexpressed in the triple-negative primary breast cancers versus double positive cases. miR-203 inhibits cell proliferation and migration, while miR-141-3p inhibits metastasis; overexpression of miR-548c-3p is associated with poor prognosis. miR-181a has been downregulated in other solid tumours, being involved in cellular transformation. Our results indicate that expression of particular miRNAs is correlated with that of specific pathways involved in tumorigenesis, invasion and metastasis, and highlights the role of miRNA in early diagnostic, prognostic and therapeutic response.

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**P1.02**

DISCOVERY OF PREDICTIVE BIOMARKERS FOR PERSONALIZED CANCER TREATMENTS THROUGH COMPUTATIONAL MODELLING OF DRUG RESPONSE MATRICES

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High throughput drug screening (HTDS) enables systematic means to profile cellular responses to a wide collection of oncology compounds, providing an unbiased strategy toward personalized treatment selection. However, this approach often results in high-dimensional data matrices, with inherent sources of noise, complicating many downstream analyses, such as detection of differential drug activity or prediction of drug sensitivity and resistance. At the same time, advances in the omics-technologies, such as sequencing and molecular profiling, has generated huge amounts of oncogenomic data; yet, the driving oncogenic signals are poorly known for many cancer types. To date, a handful of biomarkers have successfully reached the clinic and gained utility in clinical practice. Accordingly, there is an urgent need to develop more systematic strategies for making most of the quantitative data produced in the HTDS studies and for correlating drug response and molecular profiles to identify predictive biomarkers as companion diagnostics for subgrouping cancer patients and to guide next line treatment strategies for relapsed cancer patients on an individualized basis. To meet these needs, we have developed a computational pipeline from drug response scoring to biomarker identification. The pipeline is based on our novel metric to quantify drug response, called drug sensitivity score (DSS, Yadav et al., manuscript in preparation). In the next step, de-noising of the quantitative drug response matrix is carried out using computational matrix approximation techniques. Based on the normalized drug response profile of each cancer sample, together with drug target information from public resources, we next compute kinase inhibition sensitivity score (KISS, Szwajda et al., manuscript in preparation), to de-convolute the selective targets the samples are addicted to. Using the response patterns of 117 cancer cell lines to 138 chemical compounds from the Sanger Institute, we demonstrate that the target addiction profiles of the cells do not only cluster according to the cancer sub-type but also show improved correlation with the mutation profiles, compared to drug response profiles. Such experimental-computational approach provides principled means for identifying molecular biomarkers predictive of individual drug response.

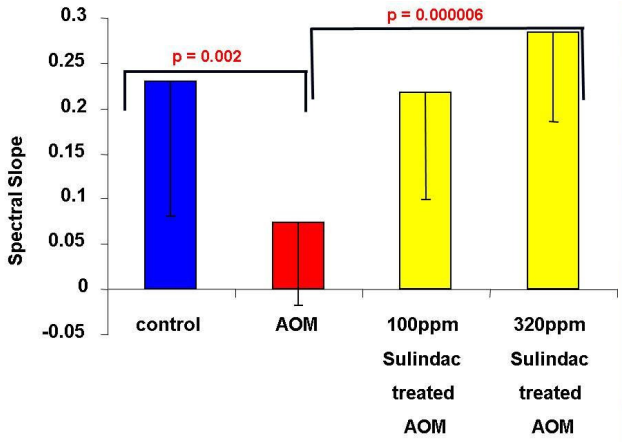
**Acknowledgements:** HBGP, FIMM and University of Helsinki Chancellor's travel grant.

**P1.03**

PERSONALIZING COLORECTAL CANCER (CRC) CHEMOPREVENTIVE STRATEGIES VIA LOW COHERENCE ENHANCED BACKSCATTERING SPECTROSCOPIC (LEBS) DETECTION OF NANO-ARCHITECTURAL CHANGES IN FIELD CARCINOGENESIS

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**Background:** Despite incontrovertible data that NSAIDS/aspirin halve CRC risk, clinical implementation of this strategy has been stymied because only ~30-50% will manifest anti-neoplastic benefit whereas the entire population is subjected to potential toxicity. Thus, a biomarker that can rapidly identify this patient subset is urgently needed. We developed a novel optical technology, LEBS, which enables detection of nano-architectural correlates of field carcinogenesis (*Gastro* 2011). Indeed, LEBS analysis of the endoscopically-normal rectal mucosa was ~90% accurate in predicting advanced adenomas elsewhere in the colon (*Cancer Res* 2009). We therefore wanted to investigate if LEBS signatures would normalize with NSAID treatment.
**Materials and Methods:** We used the azoxymethane-treated rat, a well-established model with a defined time-line (adenomas/carcinomas develop 20-40 weeks post-carcinogen, respectively). We evaluated premalignant rats (8 weeks) gavaged for 1 week with the NSAID sulindac (100 or 320 ppm) or vehicle. The LEBS parameter spectral slope (a marker of nanoscale structural distribution) was then assessed from excised colonic mucosa.
**Results:** Azoxymethane treatment profoundly decreased spectral slope consonant with neoplastic signature in humans (*Dis Col Rect* 2008). Sulindac resulted in a remarkable dose-dependent reversion in spectral slope from the premalignant (histologically-normal) epithelium (figure).




**Conclusions:** We demonstrate that NSAIDSs induced a rapid, dose-dependent normalization of nanoscale field carcinogenesis alterations as determined by our novel optical technology. We envision that serial monitoring via a minimally-intrusive rectal fiberoptic probe can enable identification of chemopreventive benefit in order to personalize therapeutics by: 1.Expeditiously discontinue/change agent in nonresponders. 2. Titrate dosage to maximize chemoprevention while minimizing toxicity.

**P1.04**

SPECTACOLOR: A COLLABORATIVE MOLECULAR SCREENING PLATFORM TO OPTIMIZE COLORECTAL CANCER PATIENT ACCESS TO BIOMARKER-LED CLINICAL TRIALS

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Newly developed targeted drugs are tested, through clinical trials, on restricted groups of patients exhibiting specific biomarkers. When the prevalence of those biomarkers is low, a high number of patients need to be screened. Recruitment of patients in a biomarker-led clinical trial requires significant effort due to the number of patients screened and the complexity and cost of such screening programs. Moreover, when various clinical trials are targeting the same population of patients, the availability of samples may be restricted and patients may lose the opportunity to enter a potential clinical trial. To avoid duplication of effort and guarantee efficient access to clinical trials for patients, the European Organization for Research and Treatment of Cancer is building a collaborative molecular screening platform, SPECTAcolor, for patients with advanced colorectal cancer. SPECTAcolor is the first European platform intended to accelerate clinical trial access by ensuring robust screening of markers that can potentially allow patients to be enrolled in open clinical trials. Thirty clinical centers in ten European countries are recruiting stage IV and advanced Stage III colorectal cancer patients. Tumor biopsies from these patients are centralized for pathological examination and screening of cancer gene alterations. Known colorectal cancer biomarkers (KRAS, BRAF, NRAS, MSI, PI3K) as well as common cancer genes are investigated using respectively certified assays and next generation sequencing technologies. By matching clinical, pathological and molecular profiles of patients to eligibility criteria of available biomarker-led clinical trials, SPECTAcolor achieves a real-time identification of eligible patients across Europe and patient-oriented parallel screening for multiple trials. In this paper, we will describe the infrastructure of SPECTAcolor which is based on an up and running network of clinical centers, a central biobanking and pathology facility, qualified assays laboratories, software to collect, trace and secure the data and algorithms to match patients in real time with trials. We will also detail the model of partnership that has been developed to engage all stakeholders in the project, including the patient organizations, the European Society of Pathology, the Sanger Institute and our funders.

SPECTAcolor is funded by the EORTC Charitable Trust through a grant from Alliance Boots.

**P1.05**

DYNAMIC CHANGES IN CIRCULATING TUMOR CELL LEVELS AS A PROGNOSTIC MARKER IN STAGE IV NON-SMALL CELL LUNG CANCER

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**Background:** We have previously reported the performance of our immuno-enrichment free High-Definition CTC (HD-CTC) assay in the enumeration and characterization of circulating tumor cells (CTCs) in lung cancer patients. In the HD-CTC assay, nucleated cells are fluorescently labeled and imaged using automated microscopy. CTCs are defined as morphologically distinct, cytokeratin positive, CD45 negative cells. Here, we investigate further how CTCs numbers and physical properties relate to prognosis in NSCLC patients.
**Methods:** The HD-CTC assay was applied for CTC enumeration in 362 blood samples collected longitudinally from 81 Stage IV NSCLC patients. Blood was collected at time 0, 3 weeks, and every 3 months thereafter. The collection protocol was restarted at disease progression, resulting in a sample number ranging between one and 12 samples per patient.
**Results:** Eighty-one patients were enrolled, with 41 patients in 1$^{st}$ line therapy, 11 in 2$^{nd}$, 17 in 3$^{rd}$, 7 in 4$^{th}$, 3 in 5$^{th}$ and 2 in 6$^{th}$ line therapy. Average age was 63, range 33 to 90; male to female ratio was 1:1.19. Eighty-six percent of patients had CTCs in at least one blood sample; CTC counts ranged from 0 to 885 CTCs/ml with a mean of 23.5 CTCs/ml across all samples. There was no difference in HD-CTC counts according to histology or line of therapy. While no correlation was found between the absolute CTC concentration and survival, it was observed that for the 26 patients that had samples collected both at the start and during first-line chemotherapy, an increase in CTC level between the two time points was significantly associated with longer progression free survival ( p=0.0007) and prolonged survival ($p$ = 0.007).
**Conclusions:** As detected with our assay, it is the change in CTC level upon entering 1$^{st}$ line chemotherapy and not the absolute CTC concentration that carries prognostic information. This observation highlights not only the importance of CTCs as prognostic biomarkers, but also that of access to serially collected specimens.

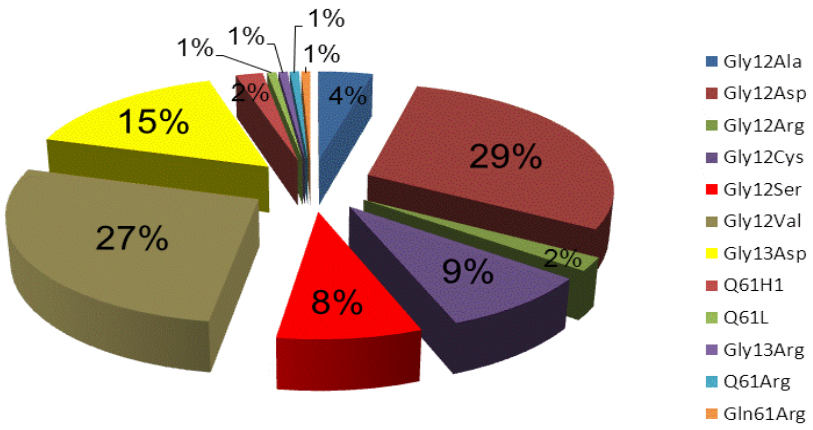
Supported by Award Number U54CA143906 from the National Cancer Institute.

**P1.06**

KRAS MUTATION DETECTION IN 334 FFPE SAMPLES

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Patients with mutations in the KRAS gene are hardly ever responsive to anti-EGFR monoclonal antibodies. Looking for mutations is essential to confirm that the tumor is mutation free, before a therapy begins. It is well established that KRAS mutation detection in CRC has a prognostic value. We collected 334 formalin-fixed paraffin-embedded (FFPE) samples from patient with metastatic colorectal cancer (CRC) for mutation detection in the KRAS gene. Twenty highly polymorphic SNPs, covering exons 12, 13 and 61 were selected. Every FFPE that was used in this study was evaluated by pathologist to determine if the section contains CRC. Hematoxylin and Eosin slides demonstrate CRC, was marked by pathologist with highlight to rich tumor cells area. Our drive was to begin with extracting DNA from area contains at least 50% of tumor cells.
Here, we show results of 334 cases that we checked for present of mutations between the years 2009 - May 2013. All cases were tested in our laboratory in the same methods as well as all DNA purification was done using the same type of kit. KRAS mutation detected by AutoGenomics INFINITI KRAS Assay. Following PCR amplification a removal of the non-incorporated dNTPs was done with mix of SAP and exonuclease I. The products were loaded on BioFilmChip microarrays (INFINITI Analyzer) together with mix containing labeled-dCTP, allele specific primer mix, extension reaction buffer and hybridization buffer. The process includes florescent label incorporation, hybrisization of analyte specific primer extension to microarray cheap, washing with wash buffer, scanning of the microarray, detection and analysis. After automatic detection of the signal from the microarray we were able to identify presence or absence (wild type) of mutations in the indicated exons.




*Frequency of mutations in the KRAS gene (%)*

334 samples were tested and the frequency in our heterogeneous population was determined. 46% of the samples were baring mutations in the KRAS gene. 29% of the mutations were Gly12Asp, 27% were Gly12Val. In 15% of our population we saw change in codon 13 to Aspartic acid.
The results are consistent with similar results in Europe, but are not in correlation to results in China.

**P1.07**

IMPROVED RESPONSIVENESS TO TEMOZOLOMIDE BY BLOCKING MIF EXPRESSION IN GLIOBLASTOMA

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Despite undeniable progress in the care and treatment of primary brain tumors, for most patients diagnosed with glioblastoma (GBM) prognosis is poor, and less than 5% of newly diagnosed GBM survive more than 5 years. Standard treatment for GBM consists of combining the alkylating agent, temozolomide (TMZ) with radiotherapy (RT) (chemoradiotherapy). Methylation of the *MGMT* promoter region of the tumour is a strong predictor of benefit with chemoradiotherapy for a subgroup of patients. However, the tumor eventually relapses. Understanding the resistance to chemoradiotherapy could facilitate the development of therapies that specifically target such resistance mechanisms.
As part of an effort to make discovery, the low molecular weight proteome of a cohort of uniformly treated GBM (surgery followed by chemoradiotherapy; n=48) was assessed using Surface Enhanced Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (SELDI-TOF-MS). High expression of the inflammatory related protein macrophage inhibitory factor (MIF) was associated with patients who were refractory to treatment and poor overall survival. MIF is a pro-inflammatory cytokine/chemokine with a broad spectrum of actions and contributes to tumorigenesis. High MIF protein expression was identified in 69% of GBM (n=230) and co-segregated with poor prognosis
The binding of MIF to its receptor complex, CD74/CD44, promotes chemoradiotherapy resistance. Inhibition of MIF with siRNA in patient-derived cells RN1 (unmethylated MGMT promoter) and BAH1 (methylated MGMT promoter) significantly improved the responsiveness of the GBM cells to TMZ. The addition of ISO-1 (1mM), an inhibitor of the tautomerase activity site of MIF to RN1 cells, also potentiated the efficacy of TMZ. Collectively, these data suggest that MIF signalling may be an additional factor that contributes to TMZ resistance in addition to the more dominant effects of MGMT. Testing patients for both MGMT promoter methylation and MIF expression may be informative of a difficult phenotype of therapeutic responsiveness.

**P1.08**

INTEGRATIVE ANALYSIS OF GENOMIC AND TRANSCRIPTOMIC DATA REVEALS THE PRESENCE OF A NOVEL MOLECULAR CLASS SHARED BY ALL CANCER ENTITIES

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Recent approaches to the classification of human tumor types have mainly been based on clinical, pathological, as well as molecular parameters. By contrast, gene expression patterns reflecting the sum of genetic aberrations in individual tumors have not been fully explored. We hypothesized that similar sets of common, tumor-specific properties exist which are independent of any histologic, clinical or single molecular parameters. In an attempt to uncover such prominent and common molecular features, we developed a novel, unbiased and integrative approach.
We first analyzed matched pairs of normal and tumor DNA of 45 renal cell carcinoma (RCC) patients, unbiased from current clinico-pathological, genetic and biological valuations, using SNP microarrays. Based on the hypothesis that genomic alterations impact on the expression of a broad array of genes, we integrated these results into a novel workflow by combining data from *in silico* data mining tools, gene expression and tissue microarrays (TMA). Finally, we used our Artificial Intelligence platform (Q-USD[TRADEMARK]) to screen for systematics identified in RCC and potential novel ones in 12 additional human tumor types encompassing more than 3000 individual samples.
We have identified five possible global constellations of genetic relationships within the tumors that we call the “Cancer States.”These Cancer States split up the entire patient cohort into up to five homologous molecular subgroups, which appear independent of any currently used pheno- or genotypic classification. Translating these results into applied tumor pathology, we demonstrate that Cancer States represent a novel view for classifying human cancer. Ongoing clinical investigations suggest Cancer States as independent predictors of overall patient survival in RCC. Tamoxifen treated breast cancer patients with Cancer State A were found to have a better prognosis compared to patients with all other Cancer States. Thus, Cancer State A clearly identifies a distinct responder subpopulation among the entire cohort of treated patients.
In summary, Cancer States are reflecting global-mRNA-represented systematics as recurrent features in human cancer. We suggest that the sum of individual molecular alterations in a given tumor represents an intrinsic roadmap to establish one out of the five Cancer States.

**P1.09**

A PHASE II STUDY AND BIOMARKER ANALYSIS OF CETUXIMAB AND FOLFIRI AS SECOND-LINE TREATMENT IN PATIENTS WITH METASTATIC GASTRIC CANCER

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**Background:** This study was conducted to explore potential biomarkers for predicting clinical outcome of cetuximab in combination with modified FOLFIRI (mFOLFIRI) and to analyze the safety of this regimen as a second-line treatment in metastatic gastric cancer patients.
**Methods**: A total of 61 patients received an initial intravenous (IV) dose of cetuximab (400 mg/m2) and weekly doses (250 mg/m2) thereafter. On day 2 of each 14-day period, patients received IV irinotecan (180 mg/m2), leucovorin (200 mg/m2), and an IV bolus dose of 5-FU (400 mg/m2) followed by a continuous infusion of 5-FU (2400 mg/m2) for 46 hours. The primary endpoint was time-to-progression (TTP).
**Findings**: The response rate (RR) was 33.3% among 54 evaluable patients. In the intention-to-treat (ITT) analysis, median TTP was 4.6 months (95% confidential interval [CI]: 3.6-5.6 months) and median overall survival (OS) was 8.6 months (95% CI: 7.3-9.9 months). In no patient, mutation of K-RAS could be detected. We further found that the levels of 4 plasma micro-RNAs are related to treatment outcome of the patients, using multivariate Cox regression analysis. The discriminant function composed of the 4 micro-RNAs can predict whether the TTP of patientswill be over 5 months or less than or equal to 5 months. The accurate rate of discrimination was as high as 87.04%.
**Interpretation:** Plasma micro-RNAs levels were identified as potential predictive biomarkers of prognosis, which warrants further investigation. Combination therapy comprising cetuximab and mFOLFIRI was well tolerated, which could potentially be used as a second-line treatment for patients with advanced gastric cancer.
**Funding:** This study was supported by Fudan University Shanghai Cancer Center; Merck KGaA Darmstadt, Germany, and National “Eleventh Five-year plan”new drug discovery major projects of P.R. China.

**P1.10**

NRF2 OVEREXPRESSION DECREASED SURVIVAL AND DIFFERENTIATION OF OVARIAN CARCINOMA THROUGH E-CADHERIN PATHWAY

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Nrf2 is a transcription factor, which involves in the progression of several human malignancies. This study was aimed to investigate the clinical significance and biological function of Nrf2 in ovarian cancer. In the present study, Nrf2 expression was immunohistochemically analyzed in 164 ovarian cancer specimens. Eighty-eight cases (49%) of the specimens examined, including 15 of 20 serous carcinomas (68.48 %), 8 of 14 endometrioid carcinomas (63.64 %), 3 of 23 mucinous carcinomas (52.17 %), and 3 of 14 clear cell carcinomas (100 %), showed Nrf2 overexpression. There is no significant difference of Nrf2 expression in mucinous tumors. Nrf2 overexpression positively correlated with advanced tumor grade ($p < 0.0001$) and had a negative correlation with E-cadherin expression ($p < 0.005$). shRNA knockdown in A2780 cell lines was also performed in the present study and the role of Nrf2 in proliferation, cell mobility and EMT (epithelial-mesenchymal transition) was then examined with biochemical assays. Nrf2 depletion in ovarian cancer cell lines inhibited cell proliferation, migration and invasion, but promoted EMT programs. Furthermore, chemotherapy in xenograft animal model was used to evaluate the expressions of Nrf2 and Snail. Our results showed that the expressions of Nrf2 and Snail in the xenograft animal model treated with chemotherapy were significantly less than in the control group. These results validated the role of Nrf2 in being a clinically relevant oncoprotein and supported that Nrf2 and E-cadherin are promising therapeutic targets in ovarian cancer.
In the future, we will create an orthotopic xenograft tumor model in nude mice by directly injecting A2780 cells into the ovarian to investigate whether genetically-modified cell line can reduce the prevalence of ovarian carcinoma in nude mice based on the changes of Nrf2 expression.
We also attempt to identify the prognosis marker by the analysis of ovarian cancer microarrays to check the efficiency of chemotherapy in ovarian cancer.

**P1.11**

LACTATE DEHYDROGENASE 5 EXPRESSION IN NON-HODGKIN LYMPHOMA IS ASSOCIATED WITH THE INDUCED HYPOXIA REGULATED PROTEIN AND PROGNOSIS

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**Background:** Non-Hodgkin's lymphoma (NHL) is a cancer that derives from cells called lymphocytes, which are part of the immune system. Lactate dehydrogenase 5 (LDH-5) is one of the major lactate dehydrogenase isoenzymes catalyzing the biochemical process of pyruvate to lactate for anaerobic acquisition energy. The purpose of this study was to investigate the level of serum LDH-5 in NHL patients and test whether LDH-5 is a prognostic marker in patients with NHL regulatied by tumor hypoxia.
**Methods:** In this study, LDH-5 levels of NHL patients (n=266) and non-NHL controls, including benign lymphadenectasis (n=30) and healthy cohorts (n=233), were detected using agarose gel electrophoresis. We also explored the expression of LDH-5 and HIF1α in NHL and benign controls by immunohistochemistry (IHC) and immunofluorescence (IF) staining, respectively. Moreover, the role of LDH-5 in the progression of NHL was assessed by using multivariate Cox analyses and Kaplan-Meier survival estimates.
**Results:** Serum concentrations of LDH-5 were significantly higher in NHL patients (10.3%) than those of benign patients (7.5%) and those of healthy controls (7.2%) ($P$<0.01, respectively). Application of LDH-5 detection increased sensitivity, identifying 53.4% of the NHL patients as positive, compared with the measurement of total LDH levels (35.5%). LDH-5 concentrations increased with age, total LDH level, clinical stage, extra-nodal sites involvement, and WHO performance status of the patients with NHL. Exposure to a hypoxic environment induced the expression of LDH-5 and its overexpression correlated with HIF1α cytoplasmic accumulation in NHL cells. In multivariate analyses, LDH-5 was an independent marker for progression-free survival (PFS) ($P$<0.001). Furthermore, the NHL patients with high LDH-5 expression had a significantly poorer prognosis compared with patients whose LDH-5 levels were lower.
**Conclusions:** The expression of LDH-5 was elevated in NHL, associating with tumor hypoxia, and also was related to unfavorable prognostic factors in the NHL patients. LDH-5 emerges as a promising prognostic predictor for NHL patients.

**P1.12**

RISK AND PREDICTORS OF SECONDARY CANCERS ASSOCIATED WITH EARLY STAGE BREAST CANCER

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**Background and Aims:** The study objective was to determine the risk of secondary primary cancers as well as to examine the predictors of secondary primary cancers in early stage breast cancer.
**Methods:** We performed a prospective cohort study of 19,580 persons recruited between 2002 and 2006 and followed up through 2008 from the national cancer registry database in Taiwan. The main outcomes were secondary primary cancer rates. Person-years of follow-up were calculated for each patient defined as the time from the newly diagnosed date to the date of the main outcome. Incidence analyses were derived by the Kaplan-Meier method. Cox proportional hazard regression models were used to estimate the multivariate-adjusted hazard ratio (HRa) with 95% confidence interval (CI) for the risk predictors of incidence of secondary primary cancer. The number of observed second primary cancer was compared to an expected number of cancers in the same age group to obtain a standardized incidence ratio (SIR).
**Results:** There were 301 secondary primary cancers during a mean follow-up period of 3.8 years (69,939 person-years of follow-up evaluation). The standardized incidence ratio showed a significant increase at the sites of stomach (SIR, 2.64, 95% CI, 1.56-4.18), colon and rectum (SIR, 1.63, 95% CI, 1.14-2.24), lung (SIR, 2.28, 95% CI, 1.57-3.20), leukemia and lymphoma (SIR, 3.52, 95% CI, 1.97-5.81), corpus uteri (SIR, 2.76, 95% CI, 1.77-4.11), ovary (SIR, 1.91, 95% CI, 1.04-3.21) and thyroid (SIR, 2.56, 95% CI, 1.67-3.75).
Women who were positive for ER and PR had significantly ($P < .01$) higher adjusted hazard ratios for secondary primary cancer incidence. The relative risk of secondary primary cancer was 0.39 (95% CI, 0.13-1.13) among women who were ER negative and PR positive, 0.76 (95% CI, 0.38-1.55) among those women who were ER positive and PR negative, and 0.59 (95%CI., 0.37-0.95) among those women who were ER negative and PR negative, as compared with women who were positive for both.
**Conclusion:** ER and PR positivity is associated with an increased risk of secondary primary cancer among breast cancer women.

**P1.13**

BONE METABOLISM MARKERS IN PATIENTS WITH OSTEOSARCOMA TREATED WITH ANTICANCER THERAPY

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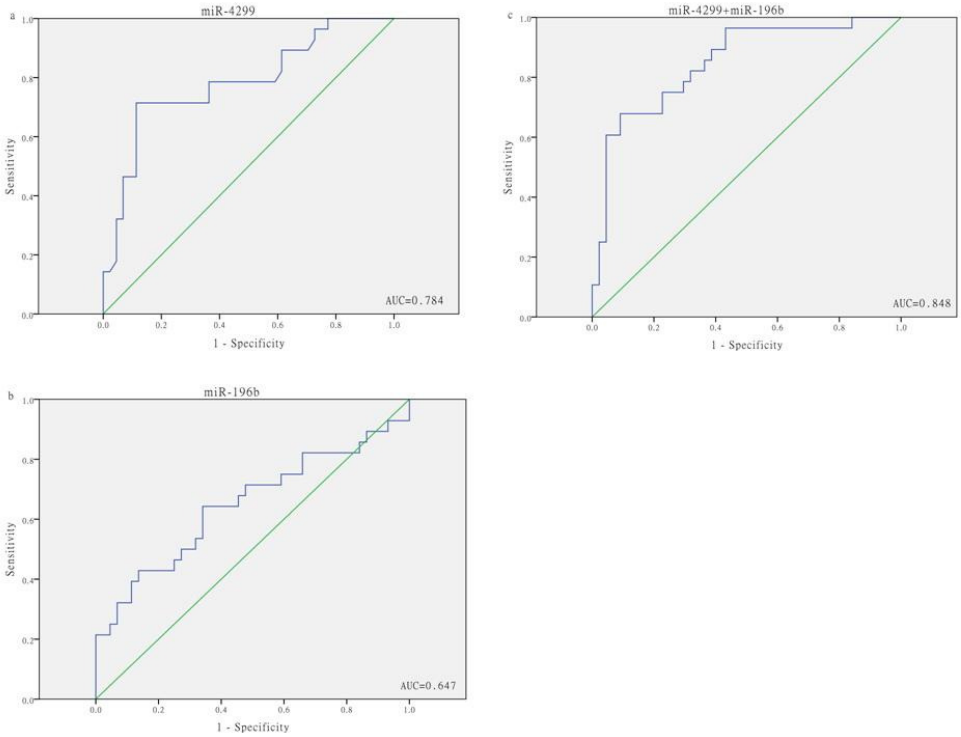
**Background:** One of the most difficult problems in pediatric oncology is the diagnosis and treatment of malignant bone tumors, that appear commonly in the second decade of life. Improvement of a comprehensive cancer treatment consisting of combination of chemotherapy and surgery enables long-term survivors. However, both the disease as well as the anticancer therapy negatively influence bone metabolism and bone mass accumulation during growth and development. Among biochemical bone markers reflecting global skeletal activity, osteocalcin (OC), C-terminal telopeptide of collagen type I (CTX), receptor activator of nuclear factor κB ligand (RANKL), and osteoprotegerin (OPG) are considered to be worth investigating. The aim of this study was to assess bone metabolism markers with special attention paid to the ratio of carboxylated OC/undercarboxylated OC and the ratio of OPG/RANKL in patients with osteosarcoma treated with anticancer therapy.
**Materials and Methods:** The study included 25 patients (median age 14.3 years) with osteosarcoma treated at the Department of Surgical Oncology for Children and Youth at the Institute of Mother and Child in Warsaw (Poland). Total bone mineral density (BMD) were performed by dual energy-ray absorptiometry (DXA) after completion of anticancer treatment. Additionally, serum biochemical bone metabolism parameters (carboxylated OC -cOC, undercarboxylated OC - ucOC, CTX, OPG, RANKL) were examined using immunoenzymatic assays. The obtained results were compared to the control group consisting of 25 healthy children (median age 14.4 years).
**Results:** In patients with osteosarcoma, the serum concentration of bone resorption marker (CTX) was reduced by about 20% compared to the controls. The ratio of cOC/ucOC as well as ratio of OPG/RANKL was about 2-fold lower in children with osteosarcoma than in the group of healthy children ($p<0.01$). Reduced z-score BMD in patients after anticancer treatment compared to the control group was observed (-1.09 versus 0.26).
**Conclusions:** Lower concentration of carboxylated osteocalcin (bone formation marker) coexisting with higher levels of bone resorption markers (CTX, RANKL) has a negative impact on bone status in patients treated for malignant bone tumors. This in turn is reflected by reduced total bone mineral density and may predispose these patients to an increased risk of osteoporosis.

**P1.14**

SPECIFIC MICRORNAS ARE NOVEL BIOMARKERS FOR CHEMORESISTANCE DETECTION OF COLORECTAL ADENOCARCINOMA

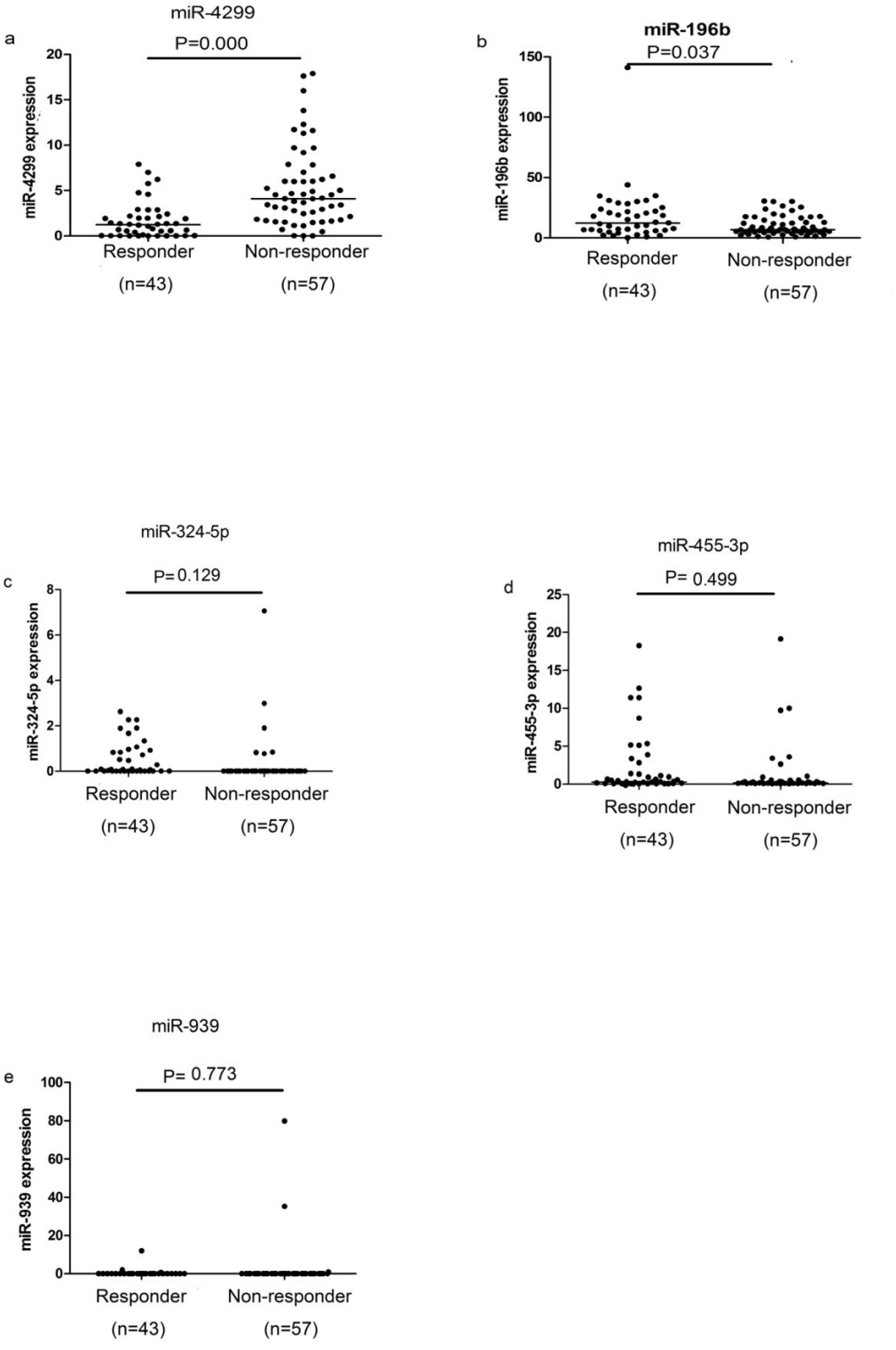
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**Purpose**: Colorectal cancer (CRC)is the most frequently occurring primary malignant tumor. Chemotherapy can reduce the risk of local and distant relapse. Therefore, it is very important to find new biomarkers that can predict chemoresistance and help in making treatment decisions.
**Methods**: In this study, we examined the expression levels of 1200 human miRNAs in 6 CRC tissues, using microRNA profiling assay arrays. A validation study was done to corroborate a subset of the results, including expression levels of miR-4299, miR-196b, miR-324-5p, miR-455-3p and miR-939, by analyzing 100 Ⅳ colorectal adenocarcinoma (not responding and responding to chemotherapy) to quantitative real-time PCR. We modeled the relationship between the expression levels of these miRNAs and the survival rate of 100 CRC patients by the Kaplan-Meier method.
**Results**: Expression profiles in CRCs suggested that 5 miRNAs were candidate markers associated with chemoresistance (capecitabine and oxaliplatin) of colorectal cancer. We found that miR-4299 and miR-196b have significant diagnostic value for chemoresistant CRC. miR-4299 yielded an AUC (area under the ROC curve) of 0.784 and miR-196b yielded an AUC of 0.647 in discriminating CRC from controls. Combined ROC analyses using these 2 miRNAs revealed an elevated AUC of 0.848 with 67.9% sensitivity and 90.9% specificity in discriminating chemoresistant CRC. A low level of miR-4299 expression and a high level of miR-196b expression are significantly correlated with good survival of CRC patients.
**Discussion**: These data suggest that miR-4299 and miR-196b have strong potential as novel biomarkers for chemoresistance detection in colorectal cancer.




*Receiver operating characteristics (ROC) curve analysis using miR-4299 and miR-196b for discriminating colorectal tumors of chemoresistance.*

(A further figure for this abstract is shown on the next page)




*Dots indicate of the relative quantification (RQ) values of miRNA expression levels, normalized by U6.*

**P1.15**

TRIAD OF HSA-MIR-155, HSA-MIR-335 AND HSA-MIR-16 AS A PREDICTOR FOR RECURRENCE RISK IN GASTRIC CANCER

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**Background:** Gastric cancer (GC) is one of the most common malignancy and primary cause of death among Chinese cancer patients. Recurrence is a major factor leading to treatment failure and the low 5-year survival rate in GC patients following surgical resection. A major challenge for improving clinical outcomes is to recognize robust signatures for the prognosis of GC. We aim to find plasma microRNAs (miRNAs) as prognostic markers that have considerable clinical value in predicting recurrence of GC.
**Patients and methods:** A total of 46 GC patients were selected for systematic analysis, 16 patients with recurrence and 30 patients without recurrence. First, we used microarray to screen miRNAs in plasma samples (n=8) of stage II patients with recurrence and stage III patients without recurrence. Next, quantitative reverse-transcriptase polymerase chain reaction assay was applied to evaluate the expression of selected miRNAs both in plasma samples and frozen fresh tissue samples(n=38).
**Results:** We have identified 19 differential miRNAs including 11 up-regulated and 8 down-regulated miRNAs in the recurrence group. Using partial least squares regression (PLSR), we further ascertained the triad of hsa-miR-155, hsa-miR-335 and hsa-miR-16 as a classifier to recognize recurrence and non-recurrence cases both in the training and test samples. Moreover, we validated this classifier in 24 frozen fresh tissues and plasma samples with consistent sensitivity and specificity. A high frequency of recurrence and poor survival were observed in GC cases with high levels of hsa-miR-155, has-miR-335 and low level of hsa-miR-16 ($P < 0.001$).
**Conclusion:** Our results suggest that the triad of hsa-miR-155, hsa-miR-335 and hsa-miR-16 as a predictor of disease progression has the potential to predict the recurrence risk in GC patients.

**P1.16**

THE PREDICTIVE ROLE OF SNP IN SELECTING FIRST-LINE CHEMOTHERAPY FOR METASTATIC COLORECTAL CANCER

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**Purpose:** To explore the predictive role of the single nucleotide polymorphisms (SNPs) of *MTHFR* and *ABCG2* in selecting an oxaliplatin-based or irinotecan-based first-line chemotherapy in metastatic colorectal cancer.
**Experimental design:** One hundred and fifty-four advanced colorectal cancer patients treated with first-line standard FOLFOX, XELOX or FOLFIRI regimen were reviewed. The SNPs of *MTHFR* and *ABCG2* were detected using gene sequencing method after DNA PCR amplification, which was extracted from peripheral blood karyocytes. Clinical characteristics and gene polymorphisms were evaluated in univariate and multivariate analysis as predictive factors for response rate(RR) and progression free survival (PFS).
**Results:** In patients carrying 2-4 genotypes of *MTHFR* 677C/C、*MTHFR* 1298 A/C or C/C、*ABCG2* 34G/G and *ABCG2* 421C/A or A/A, those who received FOLFOX/XELOX achieved a higher RR (41.7% vs. 18.8%, P=0.027) and longer PFS than FOLFIRI (8.9m vs. 7.1m, FOLFIRI: HR=1.722, 95%CI: 1.026-2.892, P=0.040, compared with FOLFOX/XELOX). On the contrary, patients carrying 0 or 1 above genotype would have better outcomes if receiving FOLFIRI chemotherapy (PFS: 9.3m vs. 6.4m, FOLFIRI: HR=0.422, 95%CI: 0.205-0.870, P=0.019, compared with FOLFOX/XELOX).
**Conclusions:** The SNPs of *MTHFR* and *ABCG2* may play a role in helping clinicians select first-line chemotherapy for advanced colorectal cancer patients.

**P1.17**

HOST GENOTYPE AND TUMOR PHENOTYPE OF CHEMOKINE DECOY RECEPTORS INTEGRALLY AFFECT BREAST CANCER RELAPSE

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**Purpose:** Chemokines play vital roles in breast cancer progression and metastasis. The primary members of chemokine decoy receptors (CDR), DRAC and D6, are expressed in breast tumors and lymphatic/hematogenous pathways and sequestrate the pro-malignant chemokines. We hypothesized that breast cancer patients carrying different levels of CDR expression in tumor and/or in host might have differing clinical outcomes.
**Experimental design:** This prospective observational study measured both somatic expression and germline genotype of DRAC/D6 in 463 primary breast cancer patients enrolled between 2004 and 2006. The endpoint was breast cancer relapse-free survival (RFS).
**Results:** In a median follow-up of 48 months, a total of 94 relapse events were observed. The 5-year RFS of all patients was 76%. There was a significant association between the co-expression of CDR with RFS (hazard ratio [HR]=0.32, 95% confidence interval [CI], 0.19-0.54). Furthermore, the co-genotype of two non-synonymous polymorphisms, DARC-rs12075 and D6-rs2228468, significantly related to relapse. Mechanistically, the variant alleles of these two polymorphisms significantly decreased by 20-30% of CCL2/CCL5 (CDR ligands) levels relative to their major counterparts. Multivariate analysis highlighted that the co-expression and co-genotype of CDR were independent predictors of RFS, with HR of 0.46 (95% CI, 0.27-0.80) and 0.56 (95% CI, 0.37-0.85), respectively. The addition of host CDR genetic information to tumor-based factors (including co-expression of CDR) improved the relapse prediction ability (P=0.02 of AUC comparison).
**Conclusion:** The host genotype and tumor phenotype of CDR integrally affect breast cancer relapse. Host-related factors should be considered for individualized prediction of prognosis.