Pyk2 and its phosphorylated form pY881 are novel prognostic markers for non-small cell lung cancer progression and patients' survival

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Background: Our previous study revealed that proline-rich tyrosine kinase 2 (Pyk2) is implicated in both anchorage-independent growth and anoikis resistance in lung cancer cells. This study aims to explore the expression and clinical significance of Pyk2 and its phosphorylated forms in non-small cell lung cancer (NSCLC).

Methods: The mRNA and protein levels of Pyk2 or cancer stem cell (CSC) markers were either examined by RT-PCR or Western blotting. An immunohistochemistry (IHC) assay was conducted to analyze the expression of Pyk2 and its phosphorylated forms in 128 NSCLC cases.

Results: The levels of Pyk2 mRNA, total protein, and its phosphorylated forms (pY402 and pY881) were higher in lung cancer lesions than in the paired non-cancerous tissues. The IHC analysis showed the levels of the Pyk2 and Pyk2 [pY881] proteins were highly expressed in 70 (54.7%) and 77 (60.2%) cases, respectively. Both Pyk2 and Pyk2[pY881] were independent prognostic factors for NSCLC patients, and had a potentially predictive role in NSCLC drug treatment. The gain and loss study of Pyk2 function revealed that Pyk2 could up-regulate CSC marker expression and enhance the transforming ability of NSCLC cells.

Conclusion: Pyk2 and phosphorylated Pyk2[pY881] are potential prognostic factors and therapeutic targets for NSCLC.
Profiling of biopsies from metastatic colorectal cancer patients identifies genomic alterations that evolve during first-line therapy

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Therapeutic resistance remains a major obstacle in metastatic colorectal cancer (mCRC) and biomarkers to guide treatment are essential to improving survival and quality of life of mCRC patients. A biopsy-driven prospective study was designed to identify biomarkers and mechanisms of resistance to a standard first-line therapy in patients with mCRC which could be useful in guiding treatment selection (QCROC-01; NCT00984048). We also hoped to recognize molecular changes over time, or resulting from the selection pressure of treatment, which could have implications for subsequent therapy. This study is ongoing and approved at 13 sites with 110 patients enrolled. Patients with mCRC receiving FOLFOX with bevacizumab consented to three needle core tumour biopsies at pre-treatment and at the time of resistance. The rate of both patient and physician acceptance of biopsies has steadily risen with time and experience. Serial bloods were also collected for proteomic analysis and circulating tumor DNA. Twenty-five biopsy samples were profiled using exome sequencing (tumor and germ line), RNAseq, low pass genome sequencing and miRNA analysis. Differential gene expression analysis revealed signatures associated with clinical response and resistance when comparing tumours obtained pre- and post-treatment. We detect changes in variant allele fraction including both depletion and enrichment of individual somatic mutations over the course of treatment, the latter of which may indicate subclonal and acquired “driver” mutations that confer therapeutic resistance. A small number of genes show recurrent evidence for changes in clonal enrichment at the time of relapse across multiple patients including TP53 and KRAS. These could also represent therapeutic targets for subsequent therapy for these patients, and as such, represent new treatment opportunities. Our findings provide insights into tumor evolution during first-line chemotherapy of mCRC that may hold clues to optimize current therapeutic decision making and identifies potential target pathways for second-line stratification of patients. This study is part of the Canadian Colorectal Cancer Consortium which is a multi-site collaboration funded by the Terry Fox Research Institute and the fonds de recherche du québec – santé.
Circulating tumor cells status (CTCs) in metastatic renal cell carcinoma (mRCC) after metastasectomy (RESORT trial)

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Background: Complete responses with targeted therapies (TTs) in mRCC are rarely achieved. Retrospective findings seem to indicate that metastasectomy (Mtx) may improve survival in selected patients (pts). The RESORT study was designed to evaluate whether Mtx followed by sorafenib (SO) may provide an additional clinical benefit in terms of disease-free survival (DFS) when compared with observation. CTCs fluctuations may reflect and anticipate treatment outcome or cancer relapse. We aimed to assess the role of CTCs as prognostic and pharmacodynamic biomarkers and to explore the association between baseline CTCs count and DFS.

Material and methods: The RESORT trial is a multicentre, open label, randomized phase II study that will be randomize 132 mRCC pts to receive SO or observation for 1 year after radical Mtx. At the time of this analysis 31 pts were enrolled. Blood samples were collected in both study arms at baseline, at 3 months after randomization, after 1 year and at disease progression (whenever it occurs). Blood samples were processed with the AdnaTest ProstateCancerSelect kit, for CTC-enrichment. CTCs were identified based on expression levels of EpCAM, MUC1 and ERBB2 using RT-multiplexPCR (BreastCancerDetect AdnaTest kit) using on purpose defined cut-offs.

Results: Out of the 22 pts enrolled at our coordinating center, 16 pts were evaluable for CTCs count. According to the count levels of CTCs at baseline and after at least 3 months from randomization we could classify pts into 4 subgroups: CTCs-negative group (6 pts), CTCs-positive turning into CTCs-negative group (6 pts), CTCs-negative turning into CTCs-positive (3 pts) and CTCs-positive group (1 pt). Interestingly, all pts in the baseline CTCs-positive turning into CTCs-negative group showed disease relapse. Four of these 6 pts were in the SO arm while 2 pts in the BSC arm. In the other subgroups there was heterogeneity in terms of either disease relapse rate and arm of treatment.

Conclusions: It may be hypothesized that the lack of predictive value of CTCs may depend on the use of SO that may down-regulate epithelial markers (e.g. EpCam) preventing capturing CTCs. Further accrual may possibly help to explain these preliminary findings and better address the role of CTCs in this setting.
Prevalence of secondary genetic modifiers on cancer drug biomarkers and implications for the clinical utility of gene-based diagnostics

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**Background:** Many tests for predictive biomarkers in tumors focus on selected known mutations or regions (e.g., hotspot panels with amplicon sequencing) or on some types of mutations (e.g., SNPs and CNAs from SNP arrays). The purpose of this study is to assess the rates and clinical impact of secondary mutations in the same predictive gene, scrutinizing the diagnostic fidelity of specific single-nucleotide variant (SNV) biomarker testing.

**Methods:** We systematically searched cancer cases for both i) the presence or absence of known predictive SNVs and ii) any high-impact mutations (HIMs) in the same gene. 1825 predictive SNV biomarkers were used in this study, from an expert-curated set of variant-drug-response relations extracted from the literature. For the HIMs, we considered any frameshifts (fs), splice site disruptions, and premature stop codons (PSCs), since they may invalidate biomarkers in their proximity. We screened 3 sets of cancer cases: a) 90 cases of endometrial cancer (EAC) from TCGA; b) 30 cases of HER2+ breast cancer (BC); and c) 209 solid tumors (ST) that were given treatment recommendations based on a NGS-based system that considers such HIMs.

**Results:** For the 3 cohorts we show the averages per sample for: i) number of detected biomarkers, ii) number of HIMs around present biomarkers (potential false positives, FP), and iii) around absent biomarkers (potential false negatives, FN). These counts include low allele frequency (AF) variants.

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<tr>
<td>BC</td>
<td>5.61</td>
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<td>EAC</td>
<td>6.04</td>
<td>0.37</td>
<td>9.27</td>
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<td>ST</td>
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In the EAC cohort, FPs occur at least 5 times with AF > 40% in cancer related genes. As for FNs, we found 13 BC cases with potentially deleterious TP53 mutations with AFs >10%. From the 209 ST cases, we have treatment recommendation information for 150. Of those, 12 patients received a recommendation based on a PSC, and one based on an fs.

**Conclusions:** Because HIMs could invalidate conclusions based on the presence or absence of standard SNV biomarkers, it is clinically important to consider them in biomarker-driven treatment decisions. Our results may explain why patients with endorsed treatment biomarkers fail to achieve the predicted clinical response and support the need for more holistic approaches to the analysis of predictive biomarkers.
Identification and validation of an 8-gene expression signature in clear cell renal cell carcinoma to predict high Fuhrman grade

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**Background:** ccRCC is the most common pathological type of renal mass. In selected patients, renal mass biopsy is used to confirm the pathological type and following treatment. Fuhrman grade used to distinguish good or poor prognosis. But the Fuhrman grade is hard to be ascertained in biopsy samples because of lack of amount of tissue.

**Materials and methods:** We use TCGA database to develop a gene expression signature to distinguish high Fuhrman grade (grade 3-4) from low grade(1-2) according to previous literature. 283 ccRCC frozen sample were retrospectively achieved from FUSCC, and 74 biopsy samples were also achieved from FUSCC prospectively.

**Results:** In our multivariate analysis of discovery set, 24 genes with a AUC 0.884 was established. In our training group from FUSCC, 283 renal mass RNA were extracted and 24 gene expression was detected by qRT-PCR and normalized to beta-actin. The –ΔCt value were used for multivariate analysis for better model. 8 genes were expressed consistently in our training group compared with discovery group with a AUC 0.823. In the biopsy validation group, the model established a good predictive capability and the AUC is 0.812.

**Conclusion:** Using TCGA database, we established a 8 gene expression signature in ccRCC to predict high Fuhrman grade, this useful tool may help diagnosis of Fuhrman grade.

Abbreviations:ccRCC, clear cell renal cell carcinoma; TCGA, the Cancer Genome Atlas; FUSCC, Fudan University Shanghai Cancer Center; AUC. Area Under roc Curve.

Figure 1. Study design. The gene expression profiles of ccRCC were investigated in 3 different phases.
Elevated expression of RNA methyl transferase BCDIN3D predicts poor prognosis in breast cancer

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**Background:** BCDIN3D is a member of the Bin3 family methyl transferase that targets the 5’ mono-phosphate of nucleic acids. Recent studies have demonstrated that BCDIN3D play important roles in MDA-MB-231 cells tumorigenic phenotypes and invasion. However, the clinical implications of BCDIN3D in breast cancer remain unclear.

**Methods:** We performed an immunohistochemistry screening for BCDIN3D using tissue microarrays constructed from 250 patients who were histologically confirmed as having invasive ductal breast carcinoma at the Fudan University Shanghai Cancer Center, aimed to evaluate the prognostic value of BCDIN3D in breast cancer patients.

**Results:** The survival analysis by Kaplan-Meier and Cox regression showed that BCDIN3D expression level served as an prognostic factor for disease-free survival ($P = 0.042$). The prognostic value of BCDIN3D was most significant in triple-negative breast cancer (TNBC) patients ($P = 0.007$).

**Conclusions:** In conclusion, our findings suggest that BCDIN3D might serve as an important prognostic factor for TNBC patients.
**COL6A1 is a new biomarker to predict metastasis of prostate cancer**

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**Background**: Effective biomarkers, specific to the advanced stage, are urgently needed for better prediction and management of prostate cancer (PCa). COL6A1 is one of the three major subunits of collagen VI which is an important extracellular matrix (ECM) protein, and is involved in multiple signaling pathways that regulate apoptosis, proliferation, angiogenesis, fibrosis, and inflammation. Recent studies demonstrated that COL6A1 was overexpressed in cancers and associated with tumor progression. In this study, we investigated the biological function and prognostic value of COL6A1 in PCa, found by our group among a panel of differentially methylated genes by DNA methylomic analysis.

**Materials and Methods**: The DNA methylation status of the COL6A1 gene was confirmed by bisulfite-sequencing PCR, and the expression pattern was determined by qRT-PCR and western blot. Cell migration and invasion were analyzed using wound healing and transwell assay. The expression level of COL6A1 protein in 223 prostate adenocarcinoma (79 localized disease/144 metastatic disease) and 40 non-cancer tissues was studied using immunohistochemistry.

**Results**: The expression level of COL6A1 gene was negatively correlated with the methylation status of its 2nd exon in PCa cell lines. COL6A1 was a positive regulator of both cell invasion and migration and correlated with the expression of MMP-9 and CXCR4. The expression level of COL6A1 protein was significantly higher in advanced stage prostate cancer with bone and/or lymph node metastasis than in early stage PCa. Overexpression of COL6A1 was associated with poor prognosis in metastatic prostate cancer patients.

**Conclusions**: The up-regulation of COL6A1 results in cell migration and invasion and may serve as a promising biomarker to predict metastasis of PCa.
Non-Hodgkin's lymphoma (NHL) is one of the leading types of cancer in Uzbekistan. The statistic figures obtained in the period from January 2009 to August 2014 at the National Cancer Research Center reveal that the most common histologic subtype of lymphomas affecting the gastrointestinal tract and colon is mucosa-associated lymphoid tissue-associated low-grade B cell lymphoma. In colon lymphoid tissue consists of isolated lymphoid follicles which are composed mainly of B lymphocytes.

The aim of the study was to identify the incidence of colon MALT and determine the immunohistochemical (IHCh) features of MALT-lymphoma localized in the colon in patients. The study included 28 patients with a diagnosis of colon maltoma, who were treated at the National Cancer Research Center. Diagnosis was established by histological and IHCh methods. Retrospective study analysis for 5 years (2009-2014) shows that colon MALT lymphoma made up 0.4 % of all cases of extranodal gastrointestinal NHL. The majority of colorectal MALT lymphomas are found in the small intestine in 8 occurrences (29%), it is followed by incidences in cecum or ascending colon – 15 cases (54%) and only 5 tumors (17%) took place in rectosigmoid colon. Differential diagnosis of colon MALT and GIST was done with CD117 and showed negative expression, for exclusion of neuroectodermal tumors we have used CD99 and the results were negative in all cases. For differential diagnosing of low-grade adenocarcinoma, resected specimens examined morphologically where slides showed lymphoepithelial lesions with diffuse proliferation of atypical lymphocytes, which IHCh stained positive for CD20, CD5, and bcl-6, but negative for CD3, CD15 and CD30. Negative IHCh staining of CD3, CD15 and CD30 totally excludes other possible subtypes of colon lymphomas.

Conclusion: Colonic MALT lymphoma is rare, however may present itself with atypical lesions and even invade the whole colon. Awareness of the expected locations of MALT lymphoma combined with knowledge of the incidence and IHCh findings leads to accurate diagnosis of lesions suspicious for this disorder and helps to differentiate this disease from other abnormalities.
P2.10

CYR61 confers sensitivity to aromatase inhibition in ER positive breast carcinoma

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Background: Studies have demonstrated that cysteine-rich 61 (Cyr61) may be involved in tumor proliferation and invasion. The role Cyr61 plays in endocrine therapy response is largely unknown.

Materials and methods: The levels of CYR61 were assayed in 35 primary breast cancer patients who received neo-adjuvant endocrine therapy for at least 3 months by immunohistochemistry staining before and after administrating letrozole, an oral non-steroidal aromatase inhibitor (AI) for the treatment of hormonally-responsive breast cancer. The expression levels of CYR61 were compared between pre-treatment and post-treatment samples by using the Wilcoxon test.

Results: In our clinical case series analysis, a significant increase in the levels of CYR61 protein was shown in post-treatment residual tumors (indicating insensitivity to endocrine therapy) compared with that in the baseline biopsy samples, which were irrespective of the efficacy of primary endocrine treatment (Figure1). In addition, the ki67 level was significantly decreased after neo-adjuvant endocrine therapy, compared with baseline expression.

Conclusions: Our study provides evidence that CYR61 may confer sensitivity to AI treatment and may offer an opportunity to target CYR61 to improve endocrine resistance in ER-positive breast cancer.
Molecular essence and treatment strategy for estrogen receptor-negative, progesterone receptor-positive, and HER2-negative breast cancer

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Purpose: The clinical significance of progesterone receptor (PgR) expression in estrogen receptor (ER)-negative breast cancer is controversial, and the clinical practice guidelines such as the 2013 St. Gallen consensus fail to categorize the ER-/PgR+/HER2- phenotype into four known intrinsic subtypes. Here, we systemically investigated the clinicopathologic features, molecular essence, and endocrine responsiveness of this phenotype.

Methods: This study included four cohorts. The first and second cohorts were from the Surveillance, Epidemiology, and End Results database (n=67,932) and Fudan University Shanghai Cancer Center (n=2,338), respectively, for clinicopathologic and survival analysis. The third and fourth cohorts were from two independent publicly available microarray datasets including 837 operable cases and 483 cases undergoing neoadjuvant chemotherapy, respectively, for clinicopathologic and survival analysis and gene-expression analysis. Characterized genes defining subgroups within the ER-/PgR+/HER2- phenotype were determined and further validated.

Results: Clinicopathologic features and survival outcomes of the ER-/PgR+ phenotype fell in between the ER+/PgR+ and ER-/PgR- phenotypes but were more similar to ER-/PgR-. Among the ER-/PgR+ phenotype, 23-33% were luminal-like and 56-65% were basal-like. We further refined the characterized genes for subtypes within the ER-/PgR+ phenotype and developed a feasible immunohistochemistry-based method that could determine the molecular essence of ER-/PgR+ using three markers: TFF1, KRT5, and EGFR. After adjustment for other prognostic factors, the three-marker defined subgroup was an independent prognostic factor for relapse (HR of 2.4, 95%CI 1.17-5.03, P=0.017). Moreover, patients with a luminal-like ER-/PgR+ subtype probably benefited more from sufficient endocrine therapy (P=0.06). In contrast, the basal-like subgroup did not benefit from endocrine therapy (P=0.61).

Conclusion: The majority of the ER-/PgR+/HER2- phenotype are basal-like and a minority are luminal-like. Detecting immunohistochemical TFF1, CK5, and EGFR helps to identify the intrinsic subgroups within this phenotype. Basal-like ER-/PgR+ tumors may avoid endocrine therapy. Further large-scale studies will be necessary to validate our findings.
Predicting 5-year overall survival and disease-free survival after curative surgery for colorectal cancer using nomograms

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**Background:** This research aimed to establish effective prognostic nomograms for colorectal cancer (CRC) after curative surgery.

**Patients and Methods:** A total of 2827 non-metastatic colorectal cancer cases, after curative surgery at Fudan University Shanghai Cancer Center (FUSCC) between January 2008 and October 2013, was used to develop nomograms to estimate the 5-year overall survival (OS) and disease-free survival (DFS), respectively. Multivariate analyses by Cox proportional hazards regressions were used to assess prognostic factors, and nomograms were constructed. The results were internally validated with bootstrap re-sampling. Performance of the nomograms was assessed by calibration curve, concordance index (C-index) and compared with AJCC staging systems (the fifth edition, the sixth edition and the seventh edition) for CRC.

**Results:** Prognostic factors of OS in the multivariate Cox model included preoperative level of CEA, preoperative level of serum CA50, T stage, LNR (lymph node ratio the number of positive lymph nodes divided by the total number of lymph nodes recovered), tumor emboli in vessels (TEIV), N stage, NLR (the number of neutrophils divided by the number of lymphocytes). Prognostic factors of DFS included preoperative level of CEA, gender, T stage, TEIV, nerves invasion, N stage, LNR, lymph node examined (LNE), which were all selected into the nomograms. The bootstrap calculated C-indexes for nomograms predicting OS and DFS were 0.80 and 0.76, which were statistically better than the C-index values of the AJCC fifth (0.68 and 0.67), sixth (0.72 and 0.71) or seventh (0.73 and 0.72) staging systems (P<0.001 for all). The two calibration curves for probability of 5-yr OS and 5-yr DFS also showed good agreement between prediction by nomograms and actual observation.

**Conclusion:** Two nomograms based on Cox proportional hazard models were developed for predicting 5-year OS and DFS of patients with CRC. Validation results revealed ideal calibration and C-index values. In the era of CRC multimodality therapy, both nomograms are reasonable tools for patients and clinicians when individualized prognosis prediction is needed.

Figure on next page.
Fig1 Nomogram for determining 5-year probability of death (A) and probability of recurrence or metastasis (B) of CRC patients. (To use the nomogram, add up the points identified on the points scale for each variable, the total points projected on the bottom.)
MiRNA-621 sensitizes breast cancer to chemotherapy by suppressing FBXO11 and enhancing p53 activity

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Introduction: The potential application of using miRNAs to predict therapeutic response to cancer treatment holds high promise but miRNAs with predictive value remain to be identified and underlying mechanisms have not been completely understood. In this study, we aimed to define a potential microRNA biomarker for predicting sensitivity to weekly paclitaxel plus carboplatin (PCb) neoadjuvant chemotherapy regimen in breast cancer patients, and to explore the involved molecular mechanisms.

Materials and methods: 81 patients with stage II or III breast cancer, who received weekly PCb regimen neoadjuvant chemotherapy, were included. Gene expression profiles from 31 patients were used to predict miRNAs that are associated with chemosensitivity. Predicted miRNA was confirmed with data from an additional 50 patients as the validation set. In parallel, in vitro and in vivo analyses were carried out to define potential molecular mechanisms regulating miRNA-dependent drug sensitivity.

Results: The level of miR-621 was significantly higher in tumors from pCR patients than that from non-pCR patients. Ectopic overexpression of miR-621 promoted apoptosis and increased chemosensitivity to paclitaxel and carboplatin both in vitro and in vivo. FBXO11 was identified as a direct target of miR-621, ectopic expression of FBXO11 attenuated increased apoptosis in breast cancer cells overexpressing miR-621 upon PTX or CBP treatment. Consistently, high FBXO11 expression significantly correlated with poor survival in breast cancer patients. Mechanistically, we found in breast cancer cells that FBXO11 interacts with p53 and promotes its neddylation, which suppressed p53 transactivity. Accordingly, miR-621-dependent FBXO11 suppression enhanced p53 activity and increased apoptosis in breast cancer cells exposed to chemotherapeutics.

Conclusions: Our study revealed that a high level of miR-621 could predict a better response to PCb chemotherapy in breast cancer patients who tend to achieve a pCR. MiR-621 enhances chemosensitivity of breast cancer cells to chemotherapy by suppressing FBXO11-dependent inhibition of p53. Therefore, miR-621 may serve as a predictive biomarker and a potential therapeutic target in breast cancer treatment.
Extracting tumor tissue immune status from expression profiles: Correlating prognosis with tumor-associated immunome

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Investigating the expression of genes in cancer-associated immune cells (immunome) is imperative for prognosis prediction. However, evaluating the expression of immune-associated genes within cancer biopsy is subject to significant inconsistencies related to the sampling methodology. Here, we present immFocus, a method for extracting immune signals from total RNA sequencing of tumor biopsies, intended for immunity depiction and prognosis evaluation. It is based on reducing the variation which biopsy preparation adds to the apparent expression levels of immune genes. We employed immFocus to normalize gene expression with an immune index using data obtained from kidney renal clear cell carcinoma biopsies. Genes that became less variable due to normalization were found to be preferentially immune-related. Moreover, immune-related genes tended to become more prognostic due to the normalization. These results demonstrate, for the first time, that whole transcriptome sequencing can be used for interrogation of a cancer immunome and for advancing immune-based prognosis.
P2.16

SPECTAlung: Screening patients with thoracic tumors for efficient clinical trial access

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Background: The identification and targeting of molecular alterations has completely changed the treatment and prognosis of lung cancer. However, designing and implementing clinical trials in small subsets of patients with a particular molecular alteration is challenging, mainly due to the lack of a uniform screening program. SPECTAlung is the first European standardized, quality-assured molecular screening program of the European Organization for the Research and Treatment of Cancer (EORTC) in collaboration with the European Thoracic Oncology Platform (ETOP) to facilitate clinical trial access for patients with thoracic tumors. It is expected to test 500 to 1000 patients each year with the overall goal of offering patients clinical trials with targeted agents.

Methods: Patients consent for their tumor tissue to be collected, centralized and processed according to defined international quality control standards at the Gustave Roussy Biobank (Villejuif, France). Next Generation Sequencing (NGS) is performed at 14 MG (Cambridge, UK) where a panel of about 360 genes is analyzed for mutations, rearrangements and gene copy number. Eligible patients are those having a pathological diagnosis of any thoracic tumor (lung cancer, malignant pleural mesothelioma and thymic malignancies) at any stage of disease, availability of tumor tissue, age at least 18 years, PS 0-2, life expectancy > 3 months, no active malignancy in the 5 years before study entry and absence of any exclusion criteria that may prevent inclusion into clinical trials. A molecular report is released to the investigator highlighting identified molecular alterations and also the trials for which the patients might be eligible. The study has been submitted to ethical committees of 15 selected highly specialized and qualified thoracic centres in 12 countries in Europe. EORTC and ETOP promote the implementation of clinical trials in molecularly selected groups of patients at the SPECTAlung centers. SPECTAlung offers innovative and attractive models of collaboration with commercial and research organizations, by improving patient access to novel therapeutic clinical trial and support the development of personalized medicine.
The roles of circulating free DNA and c-myc expression in clinical diagnosis and disease monitoring in breast cancer

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Objectives: To identify the role of circulating free DNA (cfDNA) and c-myc in diagnosis and prognosis prediction of breast cancer.

Methods: cfDNA was isolated from plasma of patients with primary breast cancer (n=83), patients with benign disease (n=22) and healthy controls (n=30) using blood DNA extraction kit, the cfDNA was detected by q-PCR, meanwhile the c-myc was amplified and followed-up determinate in 83 cases of primary breast malignancies, analyzed the roles of c-myc in disease-monitoring.

Results: The levels of cfDNA in plasma of breast cancer patients were significantly higher than those with benign breast neoplasm and healthy controls (P<0.01). With the ROC curve of the cfDNA, the cut-off value of GAPDH expression levels were set at 2-28.53 for identifying breast cancer, the sensitivity and specificity are 86.8% and 82.7%, respectively, while area under curve (AUC) is 0.898. Further, 46.0% (38/83) c-myc expression levels showed positive in the participants of 83 patients with breast cancer, however, the positive rate of c-myc levels were only 13.6% (3/22) and 6.7% (2/30) in the patients with benign disease and healthy controls. Moreover, there was 54.2% (39/72) the positive c-myc levels in the patients with cfDNA above the cut-off value (n=72). Additionally, 18 positive cases (62.1%) in the breast cancer patients with lymph nodes metastasis were significantly higher than 37.0% (20/54) in the patients with negative lymph nodes (P<0.05), also the c-myc expression levels in Ki67>30% group were elevated compared with those with Ki67<30% (P<0.01), but there was no significant difference between the patients with >2cm tumor and the patients with tumor size ≤2cm.

Conclusion: The levels of cfDNA in patients with breast cancer were higher compared with the benign and healthy controls; even there were the abnormal expressions of the c-myc in these patients, which indicate the c-myc is useful for differential diagnosis in the patients with breast cancer. C-myc levels were associated with lymph node status and cell proliferation activity, therefore, the levels of c-myc in circulating free DNA might be a novel and potential molecular biomarker for diagnosis and disease monitoring of breast cancer.
STC2 modulates head and neck squamous cell carcinoma metastasis through PI3K/AKT signaling

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Background: Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide. The major cause are the distant metastases before or after surgery. Recent studies show that stanniocalcin-2 (STC2) may play an important role in a range of human malignancies. STC2, known as mammalian peptide hormones, participates in many physiological processes such as angiogenesis, modulation of inflammatory response and tumor progression. Some studies have demonstrated that STC2 promotes metastasis, however, some studies have suggested it is a metastasis suppressor. In this study, we detected the specific role of STC2 in HNSCC.

Methods: Human HNSCC cell lines were used in animal assays and in investigating cell functional assays by CCK8, scratch-wound, transwell, flow cytometry, Western blot and immunofluorescence. Immunohistochemistry was performed to retrospectively analyze HNSCC tissue microarray, which was obtained from 300 patients between 2007 and 2012.

Results: STC2 expression was higher in AMC-HN-8 and FaDu than in CAL-27 and Tca-8113 cell lines. Overexpression of STC2 promoted cell proliferation, migration, invasion, cell anti-apoptosis, tumor formation, metastasis and arrested cell cycle at G1/S phase, but knockdown of STC2 inhibited these activities both in vitro and in vivo. For the mechanism, Western blot analyses demonstrated that STC2 promoted pAkt to accelerate HNSCC metastasis through snail by regulating vimentin, E-cardherin, and β-catenin, which could be inversed by knock down of STC2 or inhibition of pAkt by using an AKT inhibitor. Tissue microarray analysis also indicated that STC2 was significantly overexpressed in HNSCC tissues and its high expression lead to enhanced pAkt and snail expression in HNSCC patients with regional lymph node metastases (P < 0.05).

Conclusions: Our findings indicate that STC2 might play its oncogenic role and control HNSCC metastasis by regulating the PI3K/AKT pathway, which may reveal a novel mechanism to uncover the role of STC2 in HNSCC environment as well as provide a potential therapeutic strategy for HNSCC patients with high metastasis.

Key Words: STC2, pAkt, HNSCC, metastasis
P2.19

Pilot study characterizing the combined clinical actionability of multiplexed germline and tumor biomarkers

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Background: A core tenet of precision medicine is that predictive biomarkers can enhance therapeutic decision-making. Such biomarkers are of germline and somatic lineage and can be considered to exist at three different levels of established clinical validity: 1) clinically endorsed (i.e. FDA approved), 2) clinically observed (i.e. predictive effect observed in patients), or 3) translational (i.e. supported by pre-clinical or computational evidence). Little is known about the prevalence and interdependence of such biomarkers in cancer patients.

Methods: We analyzed a randomly selected set of 250 patients with solid tumors, encompassing 20 different cancer indications. Biomarkers based on SNVs (tumor variant frequency ≥ 10% and average base calling quality score ≥ 25), INDELs and fusion proteins were identified using a 613 gene NGS panel and an analytical platform that screens identified aberrations against > 5 500 peer-reviewed predictive biomarkers. Identified biomarkers were then classified according to lineage, clinical validity/evidence level, prevalence and potential functional interdependence.

Results: Predictive biomarkers were detected in 87.6% of tumors. Overall, 23.2% of the samples contained FDA endorsed biomarkers, while 69.2% contained clinically emergent biomarkers and 72% contained translational level biomarkers. Interestingly, we identified many cases where endorsed germline biomarkers predicting drug toxicity, modify the treatment conclusions drawn from somatic response biomarkers alone, due to toxicity concerns with a targeted therapy or its likely chemotherapy combination partner.

Conclusions: We have characterized the prevalence and clinical validity of predictive biomarkers in 250 patient samples. Predictive biomarkers are detected at a broad range of clinical validities. Our results confirm that somatic mutation profiling is essential but not sufficient for predicting cancer drug response, thereby supporting the need for diagnostic analysis of both germline and tumor biomarker information.
HTG EdgeSeq OBP Assay, an NGS-based gene expression assay for measuring 2,532 oncology-related genes in different sample types

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Background: We developed a new, targeted NGS-based gene expression assay that measures the mRNA levels of 2,568 genes (2,532 oncology-related biomarker genes). The HTG EdgeSeq Oncology Biomarker Panel Assay (OBP) is based on a novel derivative of HTG Molecular’s (“HTG”) quantitative nuclease protection chemistry (qNPA) that enables extraction-free quantitation of mRNA from FFPE tissue and a variety of other sample types.

Purpose: To determine (1) linearity across a wide range of sample inputs, (2) recommended sample input amounts for FFPE, cells, and extracted RNA, and (3) reproducibility of the HTG EdgeSeq OBP Assay in measuring the mRNA levels of 2,568 genes.

Methods: Lysates of 5 micron sections of FFPE tissues (lung, breast, prostate, and colon carcinoma; melanoma; 25-0.78 mm²/reaction), HCC78 cell line (7,500-469 cells/reaction), and Universal RNA (URNA; 50-1.56 ng/reaction) were used for the linearity and sample input studies. URNA (25 ng/reaction) was used to evaluate assay reproducibility across multiple runs and processors; in triplicate on one processor across three days, and across three processors. Sequencing libraries were generated from qNPA reactions and run on an Illumina MiSeq sequencing platform. The HTG EdgeSeq parser was used for post-sequencing data processing. Pearson correlation coefficients (r) were used to assess linearity and reproducibility of the assay.

Results: The R² for linearity across four concentration points for lung FFPE tissue (6.25-0.78 mm²), cell lines (1875-234 cells), and URNA (12.5-1.56 ng) were >0.97, 0.99, and 0.99, respectively. The (r) between low (1.56 mm²) and high (12.5 mm²) sample inputs for each FFPE tissue type was >0.98. The (r) for intra-run, inter-day, and inter-run reproducibility were >0.95, >0.98, and >0.98. Differential expression of tissue-specific genes was identified in the respective FFPE tissues.

Conclusions: The HTG EdgeSeq OBP Assay for a 2,568-gene panel is linear over a wide range of sample inputs, can comprehensively analyze very small, clinically relevant tissues, and is highly reproducible. The demonstrated performance of the assay in breast, lung, colon, and prostate cancer and melanoma FFPE samples enables multiplex oncology biomarker profiling of these and other malignant neoplasms.
Rational molecular assessment and innovative drug selection (RAIDs): Precision medicine in cervical cancer

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Background: Recent retrospective data (Ojesina, 2014; Wright, 2013; Doll 2015) identified major molecular alterations in cervical cancer (CC), but so far there has been no prospective assessment on patient outcome using a complete molecular profiling with quality control evaluation of treatment. The Cetuxicol (phase 2) clinical trial showed that the addition of cetuximab over a 6 week period did not improve DFS. PI3K pathway mutations in the tumor in the cetuximab treatment arm led to a worse DFS (De la Rochefordiere 2014). We are lacking prognostic and predictive biomarkers for CC treatment and there is a growing need for the development of biomarkers to follow up the course of the disease.

Methodology: RAIDs is a multidisciplinary co-operation between academic clinical centers, SMEs and translational research platforms in seven European countries. It includes: 1) a cognitive cohort study (BioRAIDs), one of the first prospective trials intended to define patient stratification for targeted therapies and 2) a targeted clinical trial using an HPV-directed vaccine in association with standard therapy. Molecular analysis on quality controlled tumor and sera samples from 500 patients enrolled in BioRAIDs combined Next Generation Sequencing (NGS) at SeqOomics (Hungary), Reverse Phase Protein array (RPPA) at Institut Curie (France) and PIK3CA mutations detection in sera at ERASMUS (The Netherlands). A bioinformatics pipeline to detect somatic mutations and state-of-the-art clustering methods were developed in the framework of the RAIDs consortium to stratify the patients into different subtypes.

Results: Exome sequencing analysis of the first quality controlled samples from the BioRAIDs patients will be presented. Major somatic alterations and DNA copy number alteration from exome sequencing profiles confirm PI3K pathway mutations to be a dominant feature in CC.

Conclusions: An algorithm will be constructed to predict patients of high risk of residual disease or recurrence following standard therapy. The identification of predictive tumor/blood based biomarkers will permit the definition of new strategies for precision medicine in CC.
Targeted RNA sequencing for simultaneous expression profiling and detection of gene rearrangements in FFPE biopsies

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Screening for rearrangements is complicated by the size of biopsy samples and the low prevalence of rearrangements. The HTG EdgeSeq system couples nuclease protection with NGS-mediated quantification, without the need of RNA extraction from FFPE. Library preparation occurs via nuclease protection followed by limited PCR cycles. We developed a HTG EdgeSeq Lung Fusion Assay, a combination lung fusion and lung cancer subtyping assay, to compare expression of the 3’ and 5’ regions of the gene and detect expression of a specific fusion junction. We tiled probes across the 3’ and 5’ regions of fusion partner genes ALK, ROS, RET, and NTRK1 and designed probes for common fusion junctions involving these genes. We also included probes to detect HER2 insertions, markers to discern between lung adenocarcinoma and squamous cell carcinomas, and tissue-type markers for lung, thyroid, breast, and melanoma. Cell lines carrying known fusion events showed that rearrangements were detectable in each line: we observed ~4-fold greater expression of the 3’ vs 5’ end of the rearranged genes and detected expression of the expected fusion junctions. NSCLC FFPE samples, with or without gene fusions, were used to demonstrate subtyping of adenocarcinoma and squamous cell carcinoma by gene expression profiles. An important question is how much heterogeneity is tolerated by the assay. By mixing two cell lines at different ratios, we detected rearrangements at a 10% fusion mix, in a total of 5K cells. Sample input for the assays can be as low as a few hundred cells or ~2 mm² of FFPE tissue. These results suggest that this assay can detect rearrangements in small biopsy samples. In two of three examples from a methods-comparison study, HTG EdgeSeq testing and FISH testing were both positive (one sample is RET-positive, one ALK-positive). The third example failed two accredited FISH testing lab attempts, due to poor sample quality. We were able to confirm the HTG EdgeSeq assay results by qPCR after isolating RNA from the FFPE block. Yet, in many clinical cases, the amount of tissue needed would preclude qPCR, resulting in a meaningful rearrangement going undetected. In summary, the HTG EdgeSeq assay is a promising technique for detecting gene rearrangements in small amounts of fixed tissue.
Background: Higher body mass index (BMI) is associated with poorer prognosis in breast cancer based on Western populations, especially in hormone-receptor (HR) positive patients. There is still little data on BMI and the outcome of Asian breast cancer patients.

Methods: Data of unilateral primary breast cancer patients who received surgery between 1999 and 2012 in our institute were retrospectively collected. BMI was computed by height and weight at diagnosis. Quartile points of BMI were selected to stratify patients: <21 kg/m², 21-22.9 kg/m², 23-24.9 kg/m², and ≥25 kg/m².

Results: 12,317 patients were included into final analysis. Higher BMI was associated with older age (P<0.001), more advanced pTNM stage (P<0.001), but not HR status (P=0.270). The median follow-up time of the cohort was 35.90 months. Survival analysis revealed that there was no difference in locoregional recurrence (LRR)-free survival (P=0.727) and distant recurrence (DR)-free survival (P=0.130) in different BMI groups. In the HR positive subgroup, the five-year DR-free survival for the four BMI groups were 92.8%, 89.8%, 90.0% and 91.7% respectively (P=0.015). Multivariate analysis suggested that DR risk was correlated with HR positive status (HR=0.647, 95%CI: 0.553-0.756, P<0.001), high tumor grade (HR=1.377, 95%CI: 1.177-1.611, P<0.001), and advanced tumor stage (HR=2.502, 95%CI: 2.245-2.787, P<0.001). However, BMI was not related to recurrence risk (HR=1.002, 95%CI: 0.938-1.070, P=0.947).

Conclusion: Our data demonstrated that BMI was not a prognostic factor for primary breast cancer in an Asian population. Further studies with longer follow-up time and survival analysis are awaited to confirm our result.