provided proof-of-principle data identifying fusion genes from a set of 6 leukaemia and prostate cancer samples. The aim is now to establish this as a robust tool that can be used to test for an extended set of known and candidate fusion genes in both a research and clinical diagnostic setting.
Materials and Methods: A combination of measurements of chimeric transcript junctions with exon-wise measurements of individual fusion partners is used. We have now gone through various literature and database sources, and our database of fusion genes now contains 559 previously reported fusion genes. A second-generation of the fusion gene microarray has been designed where about 500,000 oligos are used to interrogate each sample (NimbleGen HD2 technology, 3-plex microarrays).
Results and Conclusions: The new version of the fusion gene microarray have so far been successfully picking up known fusion genes from leukaemia cell lines, and we are currently moving into analyses of diagnostic cancer samples from leukaemia and sarcomas, as well as cell lines of various origins in search for known fusion genes in new cancer types. The method bears promise of an important complement to currently used diagnostic and research tools for the detection of fusion genes in neoplastic diseases.

1331 POSTER

Identifying the primary site using gene expression profiling in patients with carcinoma of an unknown primary (CUP): a feasibility study from the GEFCAPI

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Background: CUP are an heterogeneous family of neoplasms with a dismal prognosis, with empiric chemotherapy as the recommended treatment. The aim of this study was to evaluate the feasibility of a 500-mRNA microarray to identify the tissue of origin in patients with CUP.
Patients and Methods: Diagnostic biopsy formalin-fixed, paraffin-embedded (FFPE) specimens from 22 patients with CUP were prospectively collected. Gene expression profiling was performed using oligonucleotide microarray that contains 495 genes selected as highly differentially expressed between 49 tumor types (CupPrint®).
Results: The assay was successfully performed on specimens from 18 of the 22 patients (82%). It could not be performed because of a low RNA preservation in the remaining 4 cases. The median age was 57 years (range: 29−70 years). The median delay from tissue shipping to receipt of CupPrint® result was 11 days (range: 1−26 days). The most common tissues of origin identified were lung cancer (22%) and colorectal cancer (17%). Of note, a primary cancer which would not be adequately treated by an empiric chemotherapy regimen currently recommended in CUP (like cisplatin-gemcitabine or carboplatin-paclitaxel) was identified in about half patients: kidney cancer (1), hepatocarcinoma (1), colorectal cancer (3), head and neck cancer (2) and cholangiocarcinoma (1).
Conclusion: Gene expression profiling of FFPE biopsy specimens from patients with CUP is feasible in a reasonable delay, making it feasible in clinical practice. A phase III randomized trial is planned to compare therapy based on gene expression-suspected primary cancer versus empiric chemotherapy.

1332 POSTER

The distribution of recurrence scores in Europe and Middle East (EME) compared with the US

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Background: The Recurrence Score (RS), derived from the quantitative RT-PCR analysis of 21 individual genes, quantifies the likelihood of distant recurrence in hormonal-treated patients with estrogen receptor (ER)-positive breast cancer. We examined whether there are differences in the RS distribution between EME and the US.
Material and Methods: 2,676 tumor specimens successfully examined in the Genomic Health laboratory from January 2004 through April 2009 that were submitted by physicians from EME were included in the analyses. Quantitative expression of 16 individual cancer related genes was measured by the pre-specified 21 gene Recurrence Score assay (Oncotype DX®) on a scale from 0 to 15 (relative to reference genes), where a one unit increment is associated with a 2-fold change in expression. RS is calculated from a published equation (Paik et al, NEJM 2004) using the quantitative expression of five proliferation-related genes (CCNB1, Ki-67, MYBL2, STK15 and Survivin), four ER-related genes (ER, PR, Bcl2 and SCUBE2), two HER2-related genes (HER2 and GRB7), two invasion-related genes (CTSL2 and STMY3), and three single genes, BAG1, CD68 and GSTM1. Based on the observed distribution of expression among the tumors for each individual gene and group, we determined the range in RSs for low, intermediate and high risk patients and compared these results to those submitted by physicians in the US.
Results: The distribution of the results from EME and the US were consistent. The table shows the distribution of RSs that may be observed for low, intermediate or high risk patients between these two regions.

	EMEA (n = 2676)	US (>90,000)
RS 0−17	51%	52%
RS 18−30	37%	35%
RS >31	12%	13%

Conclusions: The distribution of the RSs in EME is similar to that in the US even though there are differences in practice pattern management of early stage breast cancer. The 21 gene Recurrence Score assay (Oncotype DX®) Oncotype DX breast cancer assay consistently identifies >50% of patients who have a RS <18. This finding is consistent with the results from the validation (Paik et al., NEJM 2004) and confirmatory studies (Habel, BC Res. 2006; Dowsett et al., SABCS 2008). Patients with a RS < 18 have been shown to have minimal, if any, chemotherapy benefit, so this assay has potential clinical utility in EME as it does in the US (Paik et al., JCO, 2006; Albain et al., SABCS 2007).

1333 POSTER

Aberrant expression of ZDHHC14 gene in human tongue squamous cell carcinoma

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Background: Molecular alterations in a number of oncogenes and tumor suppressor genes associated with metastasis of cancer could be important clues to predicting and suppressing metastasis. The aim of this study was to identify differentially expressed gene(s) among lymph node-positive (pN(+)) cases and lymph node-negative (pN(−)) cases in tongue squamous cell carcinoma (TSCC).
Patients and Methods: In this study, genetic aberrations and gene expression profiles were examined in 20 cases of primary TSCCs, paired normal oral tissues, 6 TSCC-derived cell lines, and 2 normal oral keratinocytes (NOKs). Whole genome profiling using the Affymetrix 10K SNP Mapping Array was performed on 3 pN(+) cases, 2 pN(−) cases of TSCCs and correspondence to normal tissues. In addition, we also examined mRNA expression level of the candidate gene product identified.
Results: We found that DNA copy number abnormality of chromosome 6q region is associated with metastasis of TSCCs. *ZDHHC14* is on 6q25.3, a region gained in pN(+) cases of TSCCs when compared with pN(−) cases. Quantitative real-time reverse-transcription polymerase chain reaction (RT-PCR) showed that *ZDHHC14* was over expressed in all TSCC derived-cell lines when compared with primary cultured NOKs at the mRNA level. Similar to TSCC-derived cell lines, high frequencies of *ZDHHC14* up-regulation were evident in mRNA levels of primary tumors (n = 8/20, 40%). This up-regulation also is closely associated with lymph node status (p = 0.019).
Conclusions: These results suggest that *ZDHHC14* expression may be correlated with lymph node metastasis and offer clues to the planning of new treatments such as early detection, prevention, and therapy for TSCC metastasis.

1334 POSTER

Spleen tyrosine kinase as a novel candidate tumour suppressor gene for human oral squamous cell carcinoma

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Background: Spleen tyrosine kinase (Syk) is a non-receptor type of protein-tyrosine kinase that is widely expressed in several epithelial cells. We analyzed the mutational and methylation status of the *spleen tyrosine kinase* (*Syk*) gene and both mRNA and protein levels in primary oral squamous cell carcinoma (OSCC) and OSCC-derived cell lines and examined the function of the *Syk* gene in OSCC-derived cell lines in vitro.
Material and Methods: The seven human OSCCs-derived cell lines used in this study were Ca9−22, Ho-1-N-1, HSC-2, Ho-1-u-1, HSC-4, KON and KOSC-2. Primary cultured normal oral keratinocytes (NOKs) were used as a normal control. Tumors with patient-matched normal oral tissues (when

available) were obtained at the time of surgical resection at Tokyo dental college Chiba Hospital after informed consent had been obtained from the patients according to a protocol that was approved by the institutional review board of Tokyo Dental College.
Result: Using quantitative real-time reverse transcription polymerase chain reaction, western blotting and immunofluorescence on seven OSCC-derived cell lines and NOKs, *Syk* mRNA and protein expression were commonly down-regulated in all cell lines compared with the NOKs. Although no sequence variation in the coding region of the *Syk* gene was identified in these cell lines, we found frequent hypermethylation in the CpG island region. Syk expression was restored by experimental demethylation. In addition, using a wound healing assay and in vitro invasion assay, we performed functional analysis using Syk transfected into the OSCC-derived cell lines, and they showed significant inhibition of motility and invasiveness. In clinical samples, high frequencies of Syk down-regulation were detected by immunohistochemistry (33 of 53 [62%]). Furthermore, the Syk expression status was correlated significantly ($P = 0.047$) with tumor metastasis to cervical lymph nodes.
Conclusion: These results suggest that the *Syk* gene is frequently inactivated during oral carcinogenesis and that an epigenetic mechanism may regulate loss of expression possibly leading to metastasis.

1335 POSTER

Vascular endothelial growth factor (VEGF) 936 C/T gene polymorphism is a risk factor for invasive ductal carcinoma of the breast in a sample of Croatian woman

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Cancer angiogenesis development mediatet thru vascular endothelial growth factor (VEGF) has important role in cancer metastasing and malignant growth. The aim of this study was to investigate potential relationship between VEGF gene 936 C/T polymorphism and coexistance of invasive ductal breast carcinoma in a sample of Croatian woman. In addition, there is no any data about described genetic polymorphism among Croatian feemale population. We enrolled two groups of feemale patients: 122 subjects with invasive breast carcinoma (mean age 54.1±4, range 36−81 years) and 156 healthy control subjects (mean age 57.4±6, range 32−75 years) without any history of malignancy in which the clinical evaluation including mammography and breast ultrasound did not reveal any breast pathology. Genomic DNA was isolated from peripheral venous blood, while single nucleotide polymorphism 936 C/T genotyping in the VEGF receptor was performed using PCR-RFLP methode. We have not detected any 936 T/T genotype of VEGF gene but significant association of breast cancer risk was shown in the group of woman with breast invasive ductal carcinoma compared to healthy group. Carriers of the 936 C/T genotype were more frequent among woman with invasive ductal carcinoma (46 of 122 examinies, 37.7%) than among control group (7 of 156 examinies, 4.5%). The difference was statisticaly significant ($p < 0.0001$). This study found significant evidence that examined gene polymorphism is a key factor associated with susceptibility to invasive ductal carcinoma of the breast in a sample of Croatian women.

1336 POSTER

The prognostic impact of serum angiogenic factors in renal cell carcinoma

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Background: To determine the selected serum angiogenic factors in patients with newly diagnosed renal tumor (RCC) and to correlate them with the clinical stage of the disease.
Patients: Nephrectomy or partial kidney resection was performed in 54 patients (34 men, 20 women) with newly diagnosed renal cell carcinoma (RCC). The mean age of patients was 64.4 years. The patients were divided into three groups based on the TNM classification: the 1st group – stages I + II, the 2nd group – stage III, the 3rd group – stage IV.
Method: The serum levels (collected before surgery) of GROα (CXCL1), IL-8 (CXCL8), IL-6, VEGF and bFGF were determined by the ELISA method. Clinical data (age, sex, tumor histopathology grading (HPG), disease progression and death during a-12-month follow-up period) were compared with serum levels of angiogenic factors. Kruskal-Wallis ANOVA, Mann-Whitney's test and Kolmogorov-Smirnov's test were used.
Results: In case of GROα, a significant difference was found between patients with and without progression period ($p = 0.006$), between surviving and dead patients ($p = 0.038$), between the1st and the 4th grade of HPG ($p = 0.05$), between the 2nd and the 4th grade of HPG ($p = 0.0043$) and between the 3rd and the 4th grade of HPG ($p = 0.044$). A statistically significant difference in serum concentration of IL-8 was found between patients with and without progression ($p = 0.007$), but no differences were found between the dead and surviving patients and between the various grades of HPG. A statistically significant differences in serum concentration of IL-6 were found between patients with and without progression ($p = 0.0006$) between dead and surviving patients ($p = 0.0042$), between the1st and the 4th grade of HPG ($p = 0.05$) and between the 2nd and the 4th grade of HPG ($p = 0.0041$). A statistically significant difference in serum levels of VEGF was found between patients with and without progression ($p = 0.0026$), between dead and surviving patients ($p = 0.021$). A statistically significant difference in serum levels of bFGF was found between dead and surviving patients ($p = 0.024$).
Conclusion: Out of the ten tested angiogenic factors, we found a correlation between serum levels and clinical findings for GROα, IL-8, IL-6, VEGF and bFGF. However, their use in clinical practice should be verified on a larger group of patients.
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1337 POSTER

Free light chains renal handling in patients with plasma cell dyscrasias

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Introduction: Serum immunoglobulin-free light chain (FLC) assay is a major marker in the evaluation and management of patients with plasma cell dyscrasias. In a number of these patients, anomalies in monoclonal FLC may induce tissue deposition and diseases, especially in kidney. We retrospectively analyzed the renal handling of FLC in different types of plasma cell dyscrasias.
Methods: K and L FLC concentrations were measured by nephelometry (Freelite ®, The binding site) in serum (s) and 24-h urine (u) from 85 patients. 11 patients had polyclonal hypergammaglobulinemia (H) but no monoclonal protein as detected by protein immunofixation electrophoresis (IFE) in serum and urine. 74 patients presented abnormal K/L FLC ratio (rFLC) in serum and/or urine. 22 of them had multiple myeloma (MM) with monoclonal intact immunoglobulin or FLC only, with K (MMK, n = 13) or L (MML, n = 9) light chain. 52 of them, without MM, had an increased (I) or a decreased (D) rFLC, and monoclonal protein detected by sIFE and/or uIFE.
Results: See the table.

	H	MMK	MML	I1	I2	D1	D2
CK ml/min	2.7 (0.6–6.6)	1.8 (0.9–12)	3.0 (1.9–12)	1.4 (0.4–7.3)	0.2 (0.1–0.4)$^{£}$	1.7 (0.4–4.9)	0.7 (0.2–3.9)
CL ml/min	0.6 (0.1–1.5)	1.2 (0.1–2.5)	0.8 (0.5–5.0)	0.2 (0.1–2.9)	0.1 (0.1–1.2)	0.8 (0.1–2.0)	0.02 (0.01–0.1)$^{\$}$
creatinine µmol/l	177 (112–461)	162 (63–559)	221 (72–799)	134 (60–730)	121 (61–278)	133 (64–474)	139 (60–220)

Values are median (ranges); Mann-Whitney test (significance: P < 0.05; *:MM vs H; $^{£}$: I1 vs I2; $^{\$}$:D1 vs D2).

In H, MMK and MML groups, comparable for glomerular filtration, renal clearance of K FLC (CK) and L FLC (CL) were similar indicating similar FLC renal handling in these patients with mono- or polyclonal diseases. I group was splited in 2 groups according to CK: in I2 (n = 11) as compared to I1 (n = 24) group, CK was decreased (P < 0.0001) and sFLC increased [343 (54–817) vs 47 (20–285)$^{£}$ mg/l]. D group also was splited in 2 groups according to CL: in D2 (n = 7) as compared to D1 (n = 10) group, CL was decreased (P < 0.002) and sFLC increased [213 (150–416) vs 90 (28–247)$^{\$}$ mg/l]. Low CK and CL values were also significantly decreased in I2 and D2 groups as compared to MMK, MML and H groups and as compared to patients without plasma cell dyscrasia, regardless of creatinine (not shown). Strikingly, in I2 group, 1 light chain deposition disease and, in D2 group, 2 AL-amyloidosis were diagnosed while 4 AL-amyloidosis were diagnosed in D1 group. Such low FLC renal clearance was also observed in 3 cases of MM (not included here).
Conclusions: Low FLC renal clearance might result from FLC renal tissue deposit or FLC aggregation reducing renal excretion. Whether FLC renal