three receptors expressed on multiple cell lineages. In particular, its binding to VEGFR-2 promotes activation and differentiation of neutrophils, monocytes and inhibitory myeloid cells and impairs the immune-system by affecting specific T cell subsets. We have thus investigated whether baseline inflammatory status could be predictive of favorable outcome in mCRC patients receiving bevacizumab-based biochemotherapy.
Material and Methods: This observational-retrospective-multicentric study involved 156 mCRC patients, 85 of which treated with bevacizumab-based biochemotherapy. Kaplan Meier curves, Log-Rank test and Cox's regression analysis were carried out to evaluate correlations among their PFS and VEGF, CEA, CA19.9, LDH, PCR, VES levels, lymphocyte, neutrophil, and monocyte counts and NLR before treatment.
Results: Median PFS was 10 (95% CI 8.225–11.775) months in patients who had received bevacizumab-based biochemotherapy and 6 (95% CI 4.921–7.079) months in those who had received poly-chemotherapy alone. Univariate analysis demonstrated a positive predictive values for baseline monocyte counts $\leqslant$500 cells/mmc (PFS: 12 vs 9 months, $p = 0.05$) and NLR $\leqslant$2 (PFS: 12 months vs 8 months, $p = 0.016$) only in patients who had received bevacizumab with no statistical value in those who had received chemotherapy alone ($p = 0.10$ and $P = 0.86$, respectively). A multivariate analysis confirmed in the bevacizumab group, the predictive value of NLR $\leqslant$2 (PFS: 12 vs 8 months; HR = 0.502; $p = 0.024$).
Conclusions: Baseline inflammatory status affects the treatment-related outcome of mCCR patients undergone bevacizumab-based treatments and NLR may be considered as a promising and easy-to-do biomarker able to predict their outcome.

1419 POSTER

Identification of a Protein Kinase Activity Based Biomarker Fingerprint to Predict Response to Sunitinib, Sorafenib and Pazopanib in Scirrhous Gastric Cancer Cell Lines

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Background: Scirrhous gastric cancer accounts for approximately 10% of all gastric cancers. Because of interactions with stromal tissue components, its proliferation is driven by multiple growth factors. Although a fluoropyrimidine and platinum-containing regimen has been established as the standard chemotherapy, its efficacy is not satisfactory. Chemotherapy combined with multi-targeted kinase inhibitors (MTKIs) such as sunitinib has recently been reported to be effective in a subset of patients with scirrhous gastric cancer but no predictive biomarker or companion diagnostics has been developed. The aim of this study is to identify predictive biomarkers of response to MTKIs in scirrhous gastric cancer cell lines by tyrosine kinase activity profiling with PamChip® peptide micro-arrays.
Material and Methods: For 11 scirrhous and 14 non-scirrhous gastric cancer cell lines, the growth-inhibitory effect of the MTKIs sunitinib, sorafenib and pazopanib was evaluated by the MTT assay. For assessment of their effect, protein tyrosine kinase activity of cell line lysates was measured in the absence and presence of the MTKIs on PamChip® peptide micro-arrays, containing 144 peptides derived from known human phosphorylation sites. Inhibition profiles were calculated for each MTKI by taking the log-ratio of peptide phosphorylation measured in the absence and presence of the MTKIs.
Results: In the MTT assay, cell lines HSC-39, HSC-40A, KATO-III, HSC-43 (scirrhous) and SNU-16 (non-scirrhous) were sensitive to all three MTKIs with IC50 values <1 μmol/L. Per peptide analysis showed a clear correlation between sensitivity to the MTKIs and *in vitro* inhibition of tyrosine kinase activity, with stronger response associated with more inhibition for a larger number of peptides. For all 3 inhibitors a large number of peptides were found to differ ($p < 0.01$ in a two sample t-test) between sensitive and insensitive cell lines. Partial least squares-discriminant analysis was used to correlate the *in vitro* inhibition on the PamChip® micro-arrays to the sensitivity to the MTKIs. Good predictive performance with a misclassification rate <30% was obtained with leave-one-out-cross-validation for all 3 inhibitors.
Conclusions: These data suggest that an *in vitro* assay on PamChip® peptide micro-arrays could serve as a companion diagnostic test for MTKIs to predict response in patients with scirrhous gastric cancer. Further evaluation should be considered using clinical tumour specimens.

1420 POSTER

Evaluation of Recurrence Score, Nodal Status and Traditional Clinicopathologic Metrics in a Large ER Positive Patient Cohort

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Introduction: The Oncotype DX breast cancer assay provides prognostic and predictive information for ER+ breast cancer. A wide range of Recurrence Score (RS) biology independent of traditional clinical and histopathologic variables has been reported. In a large cohort of patients with ER+, HER2− early stage breast cancer for whom the assay was ordered, the distribution of RS and RS groups was examined across joint categories of patient age, tumour size, and grade with nodal status.
Methods: 2,097 patients from Clalit Health Services (CHS) and 484 from Maccabi Health Services (MHS) in Israel had the assay ordered from 1/2008 to 6/2010. 1864 were LN-, ER+ and HER2−. 1864 were N0, 305 were Nmic, and 308 had 1−3 positive nodes (N1−3). Age, tumour size, grade, histologic subtype, and IHC PR and Ki-67 were obtained for 100%, 99%, 82%, 99%, 99% and 23% of the patients. General linear models were fit to RS to examine the joint association between nodal status and each factor with the continuous RS. Hypothesis tests were conducted at $\alpha = 0.05$. The proportions of RS scores in the Low (RS < 18), Intermediate (RS 18−30) and High (RS $\geqslant$ 31) groups were calculated for categories of these prognostic factors, by nodal status. Spearman correlation coefficients (and 95% confidence intervals) were calculated to assess the association between the RS and them.
Results: Distributions by age were: <40 yo, 3.2%; 40−49 yo, 16.3%; 50–59 yo, 29.8%; 60−69 yo, 33.7%; 70−85yo, 17.0%. By tumour size: <1 cm, 12.1%; 1−2 cm, 64.4%; >2−3 cm, 18.1%; >3 cm, 5.4%. By grade: 1, 18.0%; 2, 63.3%; 3, 18.6%. 80.2% were ductal and 12.7% lobular. 82.5% were IHC PR+ and 62.6% had IHC Ki-67 $\geqslant$10%. The distributions of patient age, tumour size, grade and PR and Ki-67 by IHC were similar among N0, Nmic and N1−3 patients. Continuous and categorical age (<50 vs. $\geqslant$50 yr), continuous and categorical tumour size ($\leqslant$2 vs. >2 cm), tumour grade, PR by IHC (both Allred Score and positive/negative status), and Ki-67 by IHC (<10% vs. $\geqslant$10%) were significantly associated with the RS ($p \leqslant 0.001$) when included in two covariate linear models with nodal status. There was no significant trend in nodal status in all cases except when analyzed with continuous tumour size ($p = 0.042$). A range of RS values was observed in each traditional prognostic factor category. The correlations between the RS and these factors are small for age and tumour size, and modest for tumour grade, PR and Ki-67, with similar results among N0, Nmic and N1−3 pts.
Conclusions: There is a range of RS biology across patient age, tumour size, grade, and IHC PR and Ki-67 as well as nodal status. The RS cannot be predicted by traditional clinicopathologic variables.

Table 1. Spearman correlation coefficients (95% CI) for continuous RS with prognostic factors, by nodal status

Traditional Prognostic factor	Nodal Status		
	N0	Nmic	N1−3
Age (years)	−0.09 (−0.13, −0.04)	−0.13 (−0.24, −0.01)	0.00 (−0.11, 0.11)
Tumour size (cm)	0.07 (0.02, 0.12)	0.15 (0.04, 0.26)	0.14 (0.03, 0.25)
Tumour grade	0.33 (0.28, 0.37)	0.24 (0.12, 0.35)	0.27 (0.16, 0.38)
PR Allred Score	−0.37 (−0.41, −0.33)	−0.29 (−0.39, −0.19)	−0.30 (−0.40, −0.19)
Ki-67 by IHC (<10, 10−14, >14%)	0.35 (0.26, 0.43)	0.05 (−0.15, 0.25)	0.34 (0.08, 0.56)

1421 POSTER

MGMT Gene Promoter Methylation Analysis by Pyrosequencing of Glial Tumours

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Background: Promoter hypermethylation of O6-methylguanine-DNA methyltransferase (MGMT) is associated with significantly longer survival in glioblastomas and low-grade gliomas treated with radiation and alkylating agents, howerver a standard method for assaying MGMT methylation level

has not been established yet. The aim of this study was to determine that pyrosequencing (PSQ) might be used to achieve MGMT promoter methylation using one to thirteen year-old archival tissue samples as a clinical biomarker in routine practice.
Material and Methods: The study set included 141 formalin-fixed paraffin-embedded (FFPE) glial tumours from the archives of the pathology department from 1997 to 2010. Quantitative measurement of MGMT gene promoter DNA methylation employed PSQ of PCR products amplified from bisulfite converted DNA.
Results: PSQ data were obtained from all 141 samples. The mean of all cases was 14.0±16.8%, and methylated cases were 32.3±14.9%. A value of percentage of methylation (PM) of each year was not significantly different ($p = 0.771$) and didn't show any linear increasing or decreasing pattern according to the age of the FFPE block. Thirty one (41.3%) out of 75 GBM were methylated. Average PM of methylated and unmethylated cases were 35.8±14.7% and 3.2±1.8% respectively ($p < 0.001$). Eight (36.4%) out of 22 anaplastic astrocytoma were methylated,with 31.8±15.5% average PM. Eight (42.1%) out of 19 astrocytoma were methylated with 22.4±15.1% average PM. A tendency was observed toward an increasing pattern of average PM with WHO grade ($p = 0.063$) in astrocytic tumours. Anaplastic oligodendroglioma showed that 4 out of 7 cases (57.4%) were methylated and average PMs were 30.0±8.5% and 4.7±1.1% in methylated and unmethylated cases, respectively. A total of five out of eight cases (62.5%) of oligodendroglial tumour were methylated, and 28.0±8.1% and 4.7±1.2% were the respective average PM of methylated and unmethylated oligodendroglial tumours ($p = 0.024$). A correlation was observed between average PM and WHO grade ($p = 0.038$) and bimodal distribution between methylated and unmethylated cases, using 9% cut-off value ($p < 0.001$).
Conclusions: The study showed that a quantitative approach for MGMT promoter methylation gave better results than the classical gel-based methylation specific polymerase chain reaction reported by various researchers on FFPE tissue samples from old archives. The PSQ method can be used for a large scale retrospective trial, but cut-off value and calculation method of the PM should be validated.

Table 1. Methylation status of glial tumours

	Methylated		Unmethylated		Total(%)	p value
	Cases, n (%)	Mean±SD	Cases, n (%)	Mean±SD		
Glioblastoma	31(41.3)	35.8±14.7	44(58.7)	3.2±1.8	75(100)	<0.001
Anaplastic astrocytoma	8(36.4)	31.8±15.5	14(63.6)	3.3±2.2	22(100)	<0.001
Astrocytoma	8(42.1)	22.4±15.1	11(57.9)	3.4±2.6	19(100)	0.001
Anaplastic oligodendroglioma	4(57.1)	30.0±8.5	3(42.9)	4.7±1.1	7(100)	0.005
Oligodendroglioma	1(100)	20.0±0.0	0	0	1(100)	
Pilocytic astrocytoma	0	0	9(100)	3.0±1.3	9(100)	
ETC	0	0	7(100)	2.7±1.1	7(100)	
Total	52	32.3±14.9	89	3.3±1.9		

1422 POSTER

Observational Cohort Study of Plasma Levels of Biomarkers in Patients With Non-small Cell Lung Cancer Treated With Bevacizumab

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Background: Antiangiogenic drugs have shown increased response rate and survival in NSCLC. No biomarkers have been described predictors of response in these patients (Pt). The aim of this study is to investigate the usefulness of the quantification of soluble VEGF, bFGF, E-selectin, and ICAM1 as biomarkers in advanced NSCLC Pt treated with Bevacizumab (BV).
Material and Method: This is an observational cohort study, concurrently, consecutively selected Pt. We included NSCLC Pt (squamous excluded), stageIV, treated with BV plus chemotherapy and without any previous antiangiogenic therapy. Plasma samples were collected before cycle 1 and after completion of cycle 2 (week 7). Levels of selected biomarkers were analyzed using commercially available ELISA kits (R&D Systems and Biolegend). We collected clinical and radiological data at the beginning, after the second cycle and at the end of treatment.
Results: We present preliminary results of 15Pt: media age was 56.9 (range 45−75; 8 male, 7 female). Basal ECOG: 0 = 9Pt; 1 = 5Pt; 2 = 1Pt. Histological subtypes were 7 adenocarcinomas, 5 large cell carcinomas and 3 not otherwise specified. Chemotherapy regimens used with BV were platinum-based doublet or monotherapy (Pemetrexed, Docetaxel) or Erlotinib. Only 4Pt presented mild adverse effects (2 hypertension; 1 bleeding; 1 thrombosis). Causes of end of BV were progression of disease (11Pt), adverse effect (2Pt), minor surgery (1Pt) and transfer to another hospital (1Pt). Measures of VEGF in the week 7 samples show consistently higher levels than baseline (207.2±52.5 vs 74.2±86.5 pg/mL). ICAM1 values also were slightly higher in the second sample (230.9±81.6 vs 209.8±63.4 ng/mL). However bFGF levels were lower in the second samples (32.3±16.5 vs 78.0±23.8 pg/mL). VEGF levels were different between Pt with clinical stability and clinical weakness (220.9±49.7 vs 152.2±7.1 pg/mL). These differences were also found in the radiological evaluation (214.5±53.1, stable vs 192.5±53.9 pg/mL, clinical weakness). At the end of the treatment, we also found differences in sICAM1 levels in both the clinical response (263.1±58.1, stable vs 142.6±75.2 ng/mL, weakness), and in the radiological evaluation (238.2±80.2, disease control vs 227.3±86.4 ng/mL, progression).
Conclusions: Changes in both VEGF and ICAM-1 levels could be used to monitor response to antiangiogenic treatment in NSCLC patients; in addition to standard clinical and radiological evaluations.

1423 POSTER

Polymorphism Analysis in the AVADO Randomised Phase III Trial of First-line Bevacizumab (BEV) Combined With Docetaxel in HER2-negative Metastatic Breast Cancer (mBC)

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Background: In the E2100 trial, single nucleotide polymorphisms (SNPs) in the promoter region of VEGF (VEGF −2578, VEGF −1154) were reported to correlate with overall survival (OS) in BEV-treated patients (pts) with mBC [Schneider, JCO 2008]. In a retrospective analysis of the AViTA trial of BEV in pancreatic cancer, SNPs in the VEGFR-1 gene appeared to correlate with efficacy [Lambrechts, ESMO 2009]. We retrospectively analysed data from the BEV–docetaxel mBC AVADO trial [Miles, JCO 2010] to explore potential relationships between genetic variability in the VEGF signalling pathway and efficacy.
Methods: In AVADO, 736 pts with HER2-negative mBC were randomised to BEV 7.5 mg/kg, BEV 15 mg/kg or placebo (PLA), each combined with docetaxel 100 mg/m^2. The primary endpoint was progression-free survival (PFS). A panel of 26 candidate SNPs in genes involved in angiogenesis and tumourigenesis was evaluated in germ line DNA using kinetic PCR. Simple Cox regression analysis (no adjustment for multiple testing) was performed to correlate genotypes with PFS and OS.
Results: Demographics and efficacy in the genetics population (n = 336) were consistent with the overall study population. In the PLA group, the VEGF −2578 C/A polymorphism correlated with PFS: each additional C allele was associated with a 23% decrease in risk of progression or death (HR = 0.727, $p = 0.043$). With BEV 7.5 mg/kg, there was an indication of potential treatment by genotype interaction ($p = 0.02$). VEGF −1154 A/G also correlated with PFS (HR = 1.4, $p = 0.015$) but only in the BEV 7.5 mg/kg arm, with a non-significant treatment interaction. No other correlations were seen between efficacy and VEGF −2578, VEGF −1154, VEGFR-1 SNP rs9582036 or other SNPs.
Discussion: Although correlations between several SNPs and efficacy have been proposed in previous studies of BEV, we observed only a weak correlation between the VEGF −2578 SNP and PFS, driven by an effect in the PLA arm. Thus, our analysis of germ line DNA samples did not confirm findings from E2100. Likewise, our data do not confirm previous findings for VEGF and VEGFR-1 SNPs. Further biomarker research to identify pts most likely to benefit from BEV continues. AVADO (NCT00333775, sponsored by Roche) has completed accrual.

1424 POSTER

Methods of Identification and Diagnosis of Lung Cancer Using Classification Systems

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Background: The goal of the study was to develop a blood test to detect non-small cell lung cancer (NSCLC) along with a robust statistical method for multimarker data mining.