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