Methods: Pts with inoperable locally advanced/metastatic gastric/gastro-oesophageal adenocarcinoma and no prior therapy were randomized 1:1 to cis for 6 cycles + cape (5-FU allowed) + bev (7.5 mg/kg) or placebo q3w until progression. The primary endpoint of overall survival (OS) was not met, but the difference in PFS between treatment arms (secondary endpoint) was significant. Clinical outcome differed by region, with increased benefit in European + Pan-American vs. Asian pts. Baseline samples for pVEGFA analysis were available from 712/774 pts, equally distributed across treatment arms. Association with PFS and OS was tested with simple/multiple regression and subgroup analyses using median value as a pre-specified cut-off.
Results: Expression of pVEGFA differed across regions, with higher levels in non-Asian pts. Pts in the control group with high pVEGFA levels had shorter OS than pts with low levels. We also observed a trend towards better effect on PFS/OS for pts with high pVEGFA expression, driven mainly by non-Asian pts (table). This effect was not seen in Asian pts.

BM population	HR PFS VEGFA low vs high *Interaction p-value*	HR OS VEGFA low vs high *Interaction p-value*
Overall (n = 712)	0.89 vs 0.64 *p* = 0.06	1.0 vs 0.73 *p* = 0.08
Non-Asia (n = 345)	0.83 vs 0.54 *p* = 0.08	0.93 vs 0.62 *p* = 0.12
Asia (n = 367)	0.91 vs 0.81 *p* = 0.68	1.02 vs 0.87 *p* = 0.56

Conclusions: pVEGFA shows potential as a prognostic and/or predictive BM candidate for PFS and OS in bev-treated AGC pts, mainly driven by non-Asian pts. The current data are similar to observations for pVEGFA from two other independent analyses in mBC (AVADO) and pancreatic cancer (AVITA, submitted ECCO 2011).

1416 POSTER

Decrease of Regulatory T Cells in Tumour-bearing Sentinel Lymph Nodes Correlates With Non-sentinel Metastases in Node-positive Breast Cancer Patients

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Background: Sentinel lymph nodes (SLNs) form a crucial front-line immune barrier against tumours. Due to the specificity and immunosuppressive activity against tumours, regulatory T cells (Treg) have been noted as a new therapeutic target in breast cancer. However, little is known about the prevalence and clinical significance of Treg in metastases as a primary therapeutic target. The aims of this study were to evaluate the prevalence and prognostic significance of Treg in tumour-bearing SLNs in accordance with the development of metastases in patients with node-positive breast cancer.
Matherial & Methods: We evaluated 30 patients as a training set including 5 patients with ductal carcinoma in situ and 25 patients showing invasive ductal carcinoma (IDC) with various nodal statuses (no metastasis, micrometastasis or macrometastasis), followed by 40 patients with SLN metastases as a validation set: 20 patients without non-SLN metastases (Non-SLN-negative) and 20 patients with non-SLN metastases (Non-SLN-positive). Treg counts were estimated using Foxp3 immunohistochemistry in the training and validation sets and CCL22 in the validation set. We then evaluated the prevalence and correlation to relapse-free survival of Treg in SLN and non-SLN.
Results: In the training set, we confirmed that Foxp3(+) Treg increased specifically in accordance with the development of tumour and lymph node metastases. In the validation set, prevalence of Foxp3(+) Treg in tumour-bearing SLN with Non-SLN-positive was decreased compared to that with Non-SLN-negative. Foxp3(+) Treg accumulation among main tumour, SLN and non-SLN was consistent with the CCL22 intensity gradient. Decreased Foxp3(+) Treg in SLN offered a predictor of patients with node-positive breast cancer.
Conclusions: Our study demonstrated that the dynamic, tumour-specific movement of Foxp3(+) Treg in SLN and non-SLN along the intensity gradient of CCL22 could identify the extent of axillary lymph node metastases, and provided a predictor of patients with node-positive breast cancer. We hope that these findings will provide a basis for a new sentinel concept and clinical applications in the diagnosis and treatment of node-positive breast cancer.

1417 POSTER

A Phase II Trial of Afatinib (BIBW 2992) in Patients With Tumours Prospectively Screened for EGFR And/or HER2 Gene Amplification or EGFR Activating Mutations

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Background: A variety of solid tumours are dependent upon EGFR or HER2 signaling pathways, and inhibition of these proteins using targeted TKIs has been a successful approach in the treatment of tumours such as breast and lung cancer. Afatinib (A), an irreversible ErbB-family receptor TKI has the potential to benefit patients with tumours driven by gene amplification/mutation of these receptors regardless of histology, suggesting the rationality to treat patients based on tumour molecular characteristics rather than primary site of origin.
Methods: A multicenter phase II trial was initiated in the US and Taiwan with an intent to identify patients across 4 different cancer categories (1: esophagogastric, 2: biliary tract or gallbladder, 3: transitional cell carcinoma of the urothelial tract and 4: gynecologic cancers) for EGFR/HER2 gene amplification and/or high polysomy ($\geqslant$4 gene copies in $\geqslant$40% of cells) by FISH, with a goal to treat approximately 48 patients (at least 12 in each category) with A. Patients with known EGFR mutations were also eligible. Other criteria included advanced stage, measurable disease by RECIST, PS 0−2, failure to respond or progression following at least one line of prior chemotherapy and no prior EGFR/HER2 TKI. Eligible patients were treated with A 50 mg, daily oral dosing until progression or undue toxicity. Tumours were tested for EGFR and KRAS mutations. Imaging assessments were performed at 6, 12 weeks, and then every 8 weeks until end of treatment. The primary endpoint was ORR per RECIST with secondary endpoints that included disease control rate, PK and safety.
Results: Trial was closed early due to recruitment challenges. Based on preliminary data pending validation, 385 patients (128, 61, 46 and 150 each in categories 1−4 respectively) were screened to identify 38 patients with FISH+ tumours [23(18%), 5(8.2%), 6(13%) and 4(2.7%) in categories 1−4 respectively] and 20 were treated (10, 1, 5 and 4 in categories 1−4 respectively) with A. Best response included 8 patients with SD, and 1 confirmed CR (HER2+ serous endometrial carcinoma). Eight patients had PD, and 3 were non-evaluable. The median treatment length with A was 83.5 days (range 9–237). Diarrhea, rash and decreased appetite were the most common AEs reported.
Conclusions: Single agent activity of A is limited yet encouraging in select patients with acceptable tolerability. Implementation of a biomarker-driven approach for patient selection in this setting is demanding.

1418 POSTER

Baseline Inflammatory Status Defined by Neutrophil to Lymphocyte Cell Count Ratio (NLR) Predicts Progression Free Survival (PFS) in Metastatic Colorectal Cancer Patients (mCRC) Undergoing Bevacizumab Based Biochemotherapy

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Background: Bevacizumab is a monoclonal antibody (mAb) to the vascular-endothelial-growth-factor (VEGF) which enhances poly-chemotherapy efficacy in mCRC patients. Unfortunately, only part of the patients will receive treatment benefit at the price of additional side-effects and considerable costs. In this context, no biomarker predictive of patients' response to bevacizumab has been identified. Bevacizumab is considered as an anti-angiogenetic agent because it subtracts free VEGF in the tumour tissue, blocking endothelial precursors' recruitment and neo-angiogenesis. VEGF depletion however, also trigger other effects; in fact, it is involved in multiple physiological mechanisms mediated by

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