Here, we combined biomarker studies from several phase III trials with bevacizumab to systematically assess whether genetic variation in VEGFA pathway genes and other genes is associated with bevacizumab-induced hypertension.
Methods: Germline DNA was available from 628 patients diagnosed with advanced primary colorectal (NO16966), pancreatic (AVITA), non-small cell lung (AVAiL), renal (AVOREN) and breast (AVADO) cancer and treated with bevacizumab. Toxicities were identified from clinical trial reports and graded according to common toxicity criteria. Overall, 113 patients had grade 1−4 bevacizumab-induced hypertension (assessed across trials with CTCAE v2−3). A total of 158 single nucleotide polymorphisms (SNPs) located in VEGFA, VEGFA-receptors (FLT1 and KDR) and other genes were selected using a SNP tagging approach and genotyped using MALDI-TOF mass spectrometry. A logistic regression on individual patient data was performed after stratification for cancer type and other covariates.
Results: Ten SNPs were associated with bevacizumab-induced hypertension ($p < 0.05$), but none of these surpassed the threshold for multiple testing ($p < 0.0003$). The three SNPs showing the strongest association ($p < 0.01$) were: rs2305949 in KDR (allelic OR 0.93, 95% CI 0.88–0.98, $p = 0.0059$), rs4444903 in EGF (allelic OR 1.06, 95% CI 1.02–1.11, $p = 0.006$); and rs1680695 in EGLN3 (allelic OR 1.07, 95% CI, 1.02–1.12, $p = 0.008$). Interestingly, rs2305949 and rs4444903 were closely linked to amino acid changes occurring on position 273 and 708 of KDR and EGF, suggesting that these changes may functionally affect both genes and thereby contribute to hypertension. Notably, rs11064560 in WNK1 was also associated with bevacizumab-induced hypertension (allelic OR 1.06, 95% CI 1.01–1.11, $p = 0.02$), thereby supporting previous observations in a limited number of patients [Frey et al. ASCO 2008].ents [Frey et al. ASCO 2008].
Conclusions: Our study represents a large genetic analysis of bevacizumab-induced hypertension using pooled data sets. The genes described warrant further investigation for their potential role in the safety profile of bevacizumab.

1413 POSTER

Expression Analysis and Study of BCL2 and the Novel Member of the Apoptotic Genes BCL2L12, as Promising Biomarkers for Monitoring of Prostate Cancer Cells' Response to Chemotherapy

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Background: Cancer continues to constitute a global public health problem. Chemotherapy is an effective approach for combating this complicated disease; however there is an urgent need of biomarkers for monitoring patients' response to it. *BCL2* gene family members, including the novel *BCL2L12* discovered from members of our research group, are known to be extensively implicated in apoptosis and their aberrant expression has been correlated with cancer progression.
Materials and Methods: The present study aims to reveal any apparent modulations in the mRNA levels of apoptotic genes belonging to the *BCL2* apoptotic gene family, including the recently identified member *BCL2L12*, upon treatment with broadly used chemotherapeutic agents. Any apparent modulations of these genes could reveal their potential role in monitoring chemotherapy response in human malignancies.
The cytostatic action of each drug was evaluated in PC3 and DU145 prostate human cancer cell lines under study, employing the MTT and trypan blue assays. Total RNA was isolated from control and treated with appropriately selected anticancer drug concentrations cells and 2 μg of it were reverse transcribed into cDNA. The expression levels of the genes under study were determined using conventional and Real-Time PCR, employing proper housekeeping genes for normalization.
Results: Increased concentrations and exposure time of the administered chemotherapeutic compounds lead to the reduction of cancer cells' proliferation efficiency. Moreover, important modulations occurred in the mRNA levels of the genes under study, fact that implicates them in distinct biochemical pathways induced upon administration of various anticancer compounds in malignant cells.
Conclusion: Our results could help towards identifying molecules which take part in the response of cancer cells to chemotherapy and could provide valuable information about the potential of novel genes that encode parts of the apoptotic machinery as valuable tools in monitoring cancer patients' response to chemotherapy, ultimately leading to more focused anticancer treatment strategies.
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1414 POSTER

Single Nucleotide Polymorphism Analysis and Outcome in Advanced-stage Cancer Patients Treated With Bevacizumab

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Background: There are no validated biomarkers predicting benefit from bevacizumab (bev) therapy. In an effort to identify such markers, biomarker studies have been integrated into several Phase III trials with bev in an attempt to correlate genetic variability in VEGFA pathway genes with the therapeutic efficacy of bev.
Methods: Germline DNA was available from 1346 subjects diagnosed with advanced primary colorectal (NO16966), pancreatic (AVITA), non-small cell lung (AVAiL), renal (AVOREN) and breast (AVADO) cancer. Overall, 628 subjects received bev. Common single nucleotide polymorphisms (SNPs) located in the hypoxia-inducible factors (HIF-1A and EPAS1), VEGFA, VEGFA-receptors (VEGFR1 and VEGFR2) and several other genes were selected using a SNP tagging approach. A total of 158 SNPs were genotyped using MALDI-TOF mass spectrometry. A meta-analysis of individual patient (pt) data was performed, after stratification for cancer type and other covariates. Genetic associations were assessed using Cox Proportional Hazard Regression for progression-free survival (PFS) and overall survival (OS).
Results: The rs4145836 SNP in EPAS1 was most significantly associated with improved PFS in both bev-treated pts (allelic HR 0.68, 95% CI 0.56–0.82, $p = 0.0001$) and placebo pts, suggesting that this SNP may be a prognostic marker for outcome independent of bev. The rs699946 SNP, located in the VEGFA promoter, was associated with improved PFS in bev-treated subjects with an allelic HR of 1.27 (95% CI 1.08–1.49, $p = 0.003$). No effect was seen in placebo subjects, suggesting that rs699946 may be a predictive marker for favourable outcome with bev treatment. The nearby rs699947 SNP in VEGFA, which has previously been associated with bev treatment outcome in breast cancer [Schneider et al. 2008], was not associated with a PFS advantage in our study. In terms of OS, the rs12505758 SNP in VEGFR2 was most significantly associated with improved OS in bev-treated pts (allelic HR 1.50, 95% CI 1.21–1.85, $p = 0.0002$). No effects for rs12505758 were seen in placebo pts.
Conclusions: Our study represents a large genetic analysis of SNPs in correlation to bev outcome, based on pooled data sets. The observed associations suggest certain genetic loci as potential markers for favourable prognosis, regardless of bev treatment, and prediction of benefit from bev. Further studies will be necessary to assess the potential clinical value of these preliminary associations.

1415 POSTER

Blood Plasma VEGFA Analysis in the AVAGAST Randomized Study of First-line Bevacizumab (bev) + Capecitabine/Cisplatin (cape/cis) in Patients (pts) With Advanced Gastric Cancer (AGC)

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Background: Recent data suggested that high plasma VEGFA (pVEGFA) levels might predict progression-free survival (PFS) benefit in bev-treated pts with metastatic breast cancer (mBC) [AVADO study, Miles et al. SABCS 2010]. Current data in lung, renal, and colorectal cancer indicate a more prognostic than predictive role for pVEGFA [Bernaards et al. ASCO 2010]. The AVAGAST study included prospective evaluation of pVEGFA as a biomarker (BM).

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