1134 POSTER

Epithelial-to-Mesenchymal Transition and Ovarian Carcinoma: New Insights in Response Prediction to Standard Treatment

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Background: Ovarian carcinoma is the fourth most common cancer in women and the leading cause of gynaecological cancer-related mortality in the western countries. The management of ovarian cancer involves citorreductive surgery followed by a combination chemotherapy regimen that includes paclitaxel and a platinum compound (carboplatin or cisplatin). Outcome is significantly improved with this regimen, thus 40 to 50% of patients achieve a complete clinical remission. There is a crucial need to identify new biomarkers predictive of response to the standard treatment. Recently, tumour microenvironment has become an attractive target in gynaecological malignancies. In ovarian carcinoma, acquisition of invasiveness is accompanied by the loss of the epithelial features and the gain of a mesenchymal phenotype, a process known as epithelial-mesenchymal transition (EMT). In pre-clinical settings, EMT is related to tumour progression and resistance to chemotherapy. We focus on a selected group of genes related to this process, to identify a gene expression profile as a predictive marker for response to treatment.
Materials and Methods: Patients (n = 84) were staged according to the International Federation for Gynecology and Obstetrics (FIGO) classification. Optimal debulking was defined as $\leqslant$1 cm (diameter) residual disease. A complete response (CR) was defined as absence of all clinical/radiographic evidence of disease. Specific TaqMan Gene Expression assays for 43 genes were selected and gene expression was determined by qRT-PCR with TaqMan Low Density Arrays (Applied Biosystems). We applied a normalization factor based on the geometric mean of four housekeeping genes, selected by Genorm Software. A Logistic regression analysis was used to build multiple models based on the combination of significant genes, selected by the Akaike Information Criterion and the Harrell index. The accuracy of the model was determined by using the receiver operating characteristics (ROC). SAS 9.1, Enterprise Guide 3.0 and SPSS (version 9.0; SPSS Inc Chicago, IL, USA) packages were used for statistical tests. Leave-one-out cross validation to avoid over-estimation was performed using R language version 2.2 with the Design Software package version 2.0.
Results: We identify a 6 gene expression profile related to EMT. The area under curve (AUC) were 0.868 (0.791–0.946) and 0.809 (0.716-.902) after leave one out cross-validation, both with a p value <0.001. In a multivariate analysis, both the profile and the debulking status were independently associated with clinical response.
Conclusions: We found a 6 gene expression profile, related to EMT process with predictive value for response in advanced ovarian carcinoma. Even thought, an independent validation is necessary to confirm these data.

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Proteomic Anlaysis of Differenially Expressed Proteins in Patients With Metastatic Colorectal Cancer Responding to Bevacizumab

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Objective & Background: Treatment of patients with metastatic colorectal cancer includes chemotherapy and a monoclonal antibody (Cetuximab or Bevacizumab). Patients who have k-ras mutated tumours are given Bevacizumab. However, no biomarker exists to determine those patients who will respond to this targeted treatment. The objective of this study was to investigate the differential protein expression between patients who do and do not respond to bevacizumab and compare this with normal controls.
Methods: Serum from 24 patients diagnosed with metastatic colorectal cancer and 11 normal controls were collected pre-treatment and stored. All patients received Bevacizumab along with chemotherapy. Progression free and overall survival data was collected on all patients. Serum was depleted of high abundant proteins and protein expression analysed using fluorescence two-dimensional differential in-gel electrophoresis (2 D-DIGE). Gels were scanned using a Typhoon 9410 Variable Mode Imager (GE Healthcare). The images were cropped (ImageQuant 5.2, GE Healthcare) and exported into Progenesis SameSpots v3.3 (Nonlinear Dynamics, UK) for quantitative analysis.
Results: 80 spots were differentially expressed between responders and non-responders of Bevacizumab, and of these, 10 spots had significant power (80%) to be carried forward for subsequent mass spectrometry analysis. 214 spots were differentially expressed between cancer patients and normal controls ($p < 0.05$) and 99 of these had power >0.8.
Conclusion: There is a significant difference in protein expression patterns between responders and non responders to Bevacizumab. Mass spectrometry is currently identifying these proteins which could used as potential biomarkers of response to Bevacizumab and help us understand resistance to this targeted therapy.

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Association of Common Variants of GSTP1, GSTA1 and TGFβ1 Genes With the Risk of Radiation-induced Subcutaneous Fibrosis in Breast Cancer Patients

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Background: In order to provide new insights on the genetic basis of normal tissue radiosensitivity, we evaluated the association between eight polymorphic variants located in six genes related to DNA repair mechanisms, oxidative stress and fibroblast proliferation (XRCC1 Arg399Gln, XRCC1 Arg194Trp, TP53 Arg72Pro, GSTP1 Ile105Val, GSTA1 C-69T, eNOS G894T, TGFβ1 C-509T, TGFβ1 T869C) and the risk of subcutaneous fibrosis in a retrospective series of patients who received radiotherapy after breast conserving surgery.
Methods and Materials: Subcutaneous fibrosis were scored according to the LENT-SOMA scale in 257 breast cancer patients who underwent surgery plus adjuvant radiotherapy. Genotyping was conducted by PCR-RFLP analysis on genomic DNA extracted from peripheral blood. The association between genetic variants and the risk of moderate to severe fibrosis was evaluated by binary logistic regression analysis.
Results: Two hundred thirty-seven patients were available for the analysis. Among these, 41 patients (17.3%) developed moderate to severe fibrosis (G2−3) while 196 (82.7%) patients displayed no or minimal fibrotic reactions (G0−1). After adjustment of confounding factors, GSTP1 Ile105Val (OR: 2.660, 95% CI: 1.169–6.051, P = 0.019), GSTA1 C-69T (OR: 3.109, 95% CI: 1.150–8.405, P = 0.025) and TGFβ1 T869C (OR: 0.281, 95% CI: 0.085–0.926, P = 0.036) polymorphisms were found to be significantly associated with the risk of G2−3 radiation-induced fibrosis. In the combined analysis, carriers of 3 risk genotypes were found to be at higher odds to develop G2−3 fibrosis compared to patients with 2 risk genotypes (OR: 3.986, 95% CI: 1.420–11.188, P = 0.009) or 0−1 risk genotype (OR: 8.235, 95% CI: 2.598–26.096, P = 0.0003).
Conclusions: These results suggest that functional variations in genes involved in oxidative stress response and fibroblast proliferation may modulate the development of radiation-induced fibrosis in breast cancer patients. Results of the combined analysis support the notion that approaches based on the combination of different genetic markers have the potential to predict normal tissue responses.

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Is MMP-9 Q279R a Possible Marker of Prognostic in Non Small Cell Lung Cancer?

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Background: Lung cancer is the leading cause of death by cancer in the world, originating about 17.5% of total deaths from cancer (1.18 million). The extracellular microenvironment is a dynamic entity and provides regulatory signals on an intricate network of pathways that include cell adhesion, differentiation, division and apoptosis.
Matrix metalloproteinases (MMP), comprise a family of proteolytic enzymes which are involved in the regulation of various cell behaviours, including cancer cell growth, differentiation, apoptosis, migration, invasion, and the regulation of tumour angiogenesis and immune surveillance. MMP-9 overexpression contributes to cancer development and progression. A nonsynonymous A to G transition in exon 6 of *MMP9* leading to a substitution of arginine by glutamine at position 279 (*MMP9 Q279R*; rs 17576) has been shown to affect the substrate binding capacity. The aim of this study was to evaluate the influence of this polymorphism in the overall survival of non-small cell lung cancer (NSCLC) patients.
Material and Methods: Caucasian patients(n = 156) admitted to the Portuguese Institute of Oncology of Porto (IPO-Porto), Portugal, with cytological or histological confirmed NSCLC, have been prospectively

recruited to the study (1997–2010). Patients were divided according to histological types in two major groups: epidermoid NSCLC (n = 61) and non-epidermoid NSCLC (n = 95). DNA was extracted from peripheral-blood samples. The *MMP-9 Q279R* was genotyped by Real-Time PCR. Overall survival (OS) was the endpoint of this analysis and was calculated from the date of diagnosis to date of death of the patient. Survival data were analyzed according to *MMP-9* polymorphisms' genotypes.
Results: The *MMP-9 Q279R* polymorphism was significantly associated with overall survival in the non-epidermoid subgroup. Patients with genotypes carrying the G allele (AG/GG) had a statistically significant diminished survival when compared with patients with AA genotype (18.5 months and 28.7, respectively; P = 0.019).
Conclusion: Our results suggest that *MMP-9 Q279R* polymorphism is associated with a decreased overall survival in non-epidermoid NSCLC patients. In the era of pharmacogenomic profiles and directed therapies, it would be important to conduct further functional studies are to clarify the role of this polymorphism in MMP-9 expression and how it conditions tumour progression, in order to better understand the observed effect.

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Neutropenia as a Biomarker of Sunitinib Efficacy in Patients (Pts) With Gastrointestinal Stromal Tumour (GIST)

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Background: KIT is key to hematopoietic cell growth and development, and potent inhibitors of KIT signaling are likely to exhibit some degree of myelotoxicity through on-target mechanisms. We retrospectively investigated the association between myelosuppression and efficacy endpoints in sunitinib-treated pts with GIST from four clinical trials (RTKC-0511–013, NCT00075218, NCT00137449 and NCT00372567; Pfizer).
Materials and Methods: Analyses included data from a total of 416 pts with GIST, of whom 325 received sunitinib on an intermittent schedule (283 at 50 mg/day on a 4-week-on/2-week-off schedule) and 91 received sunitinib at 37.5 mg on a continuous daily dosing schedule. Median TTP, PFS and OS were estimated by Kaplan–Meier (KM) methods and compared between pt subgroups using the log-rank test. Multivariate and time-dependent covariate analyses were performed, the latter to address potential bias from longer drug exposure. Myelosuppression was graded using CTCAE v 3.0.
Results: In KM and multivariate analyses, neutropenia grade $\geqslant$2 during treatment was associated with significantly longer TTP, PFS, and OS (Table 1). Thrombocytopenia grade >1 was associated with significantly longer TTP and PFS. Hemoglobin $\leqslant$ the lower limit of normal (LLN) during treatment was significantly associated with longer TTP and showed a similar trend for longer PFS in KM analysis; both were significant in multivariate analysis. However, in time-dependent covariate analysis, only the associations between neutropenia grade $\geqslant$2 and PFS and OS showed statistical significance. Baseline neutrophil and platelet counts < median and baseline hemoglobin $\geqslant$ median were associated with significantly longer OS in KM analysis only (data not shown). Analyses of safety endpoints associated with hematologic parameters will be presented.

Table 1. Association between myelosuppression and efficacy outcomes

Efficacy endpoint	Median time to progression/ survival event (mo)	Median time to progression/ survival event (mo)	P	Multivariate analysis, HR (P*)	Time-dependent covariate analysis, HR (P*)
Neutropenia during treatment (AE data)					
	Gr $\geqslant$2 (n = 164)	Gr <2 (n = 252)		$\geqslant$/< Gr 2	$\geqslant$/< Gr 2
TTP	9.2	5.3	0.0003	0.585 (0.0001)	0.812 (0.108)
PFS	14.7	10.6	<0.0001	0.523 (<0.0001)	0.768 (0.036)
OS	25.2	15.7	<0.0001	0.598 (0.001)	0.599 (0.0006)
Thrombocytopenia during treatment (AE data)					
	Gr >1 (n = 42)	Gr $\leqslant$1 (n = 374)		>/$\leqslant$ Gr 1	>/$\leqslant$ Gr 1
TTP	10.8	6.7	0.001	0.457 (0.001)	0.710 (0.160)
PFS	10.1	6.2	0.001	0.505 (0.001)	0.782 (0.279)
OS	28.1	19.0	0.088	0.690 (0.141)	0.829 (0.429)
Hemoglobin during treatment (lab data)					
	$\leqslant$ LLN (n = 315)	> LLN (n = 85)		$\leqslant$/> LLN	$\leqslant$/> LLN
TTP	7.8	6.7	0.047	0.655 (0.003)	0.897 (0.401)
PFS	6.9	6.2	0.059	0.674 (0.004)	0.942 (0.636)
OS	20.1	19.7	0.301	0.801 (0.231)	1.241 (0.175)

Gr: grade; *Wald chi-square test

Conclusions: Neutropenia may be a previously unrecognized biomarker of sunitinib efficacy significantly associated with improved TTP, PFS and OS in pts with GIST. These results require validation in prospective trials. Hematologic parameters should be monitored closely during sunitinib treatment.

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Asthenia and Fatigue as Potential Biomarkers of Sunitinib Efficacy in Metastatic Renal Cell Carcinoma

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Background: Asthenia and fatigue (A/F) are commonly reported adverse events in patients with metastatic renal cell carcinoma (mRCC) treated with sunitinib. In a randomized phase III trial of treatment-naïve mRCC patients, sunitinib showed superior progression-free survival (PFS) over interferon-alfa (11 vs. 5 months; P < 0.001), with a median overall survival (OS) of 26.4 months (Motzer, 2009). This established sunitinib as a reference standard of care for advanced RCC. Effective management of patients' adverse events may help maximize clinical benefit.
Materials and Methods: We retrospectively investigated correlations between A/F and efficacy endpoints using pooled data from 770 sunitinib-treated mRCC patients from five clinical trials (NCT00054886, NCT00077974, NCT00083889, NCT00338884, NCT00137423; Pfizer). Patients received sunitinib 50 mg/d on a 4-weeks-on-2-weeks-off schedule (n = 544; 71%) or 37.5 mg continuous daily dosing (n = 226; 29%). Adverse events were recorded regularly and graded according to CTCAE v 3.0. Median time to tumour progression (TTP), PFS and OS were estimated using Kaplan–Meier methods and compared between patients with and without A/F using a log-rank test. Multivariate analysis was performed using age, gender, race, baseline Eastern Cooperative Oncology Group performance status, time from diagnosis to treatment, relative dose intensity, lactate dehydrogenase, serum hemoglobin, corrected serum calcium, and baseline blood pressure as covariates. Time-dependent covariate analysis was performed to address potential bias from longer drug exposure. Landmark analyses were used to compare outcomes in patients with or without A/F after 6 and 12 weeks of treatment.
Results: Of 770 patients, 583 (76%) developed A/F of any grade, compared with 187 (24%) who did not. Patients who developed any-grade A/F had significantly better TTP (11.1 vs. 6.5 months), PFS (10.9 vs. 6.4 months), and OS (26.2 vs. 15.0 months) than patients who did not develop A/F (all P < 0.0001). Multivariate analysis showed that sunitinib-associated A/F was a significant predictor of improved outcome for all endpoints (P < 0.0001). However, these results were not confirmed statistically in time-dependent covariate or landmark analyses. Analyses investigating the impact of A/F severity on outcome are in progress.
Conclusions: In patients with mRCC, sunitinib-associated A/F seems significantly and independently associated with improved clinical outcomes (TTP, PFS and OS). Since time-dependent covariate and landmark analyses supported the hypothesis that A/F may develop in patients who have longer drug exposure, the value of A/F as an early predictor of efficacy requires further analysis. This is the first reported link between drug-associated A/F and efficacy, and validation in prospective studies is warranted.

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Asthenia and Fatigue as Predictors of Sunitinib Efficacy in Gastrointestinal Stromal Tumour (GIST)

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Background: Sunitinib is an established treatment for imatinib-resistant/intolerant GIST. Asthenia and fatigue (A/F) are commonly reported side effects of sunitinib that may lead to dose reduction, potentially affecting patient outcome. Identification of a significant association between A/F and efficacy could have implications for patient management.