trials into 3 prognostic groups ($p < 0.0001$): (A) Good prognosis (score 0−1, median overall survival (OS) 63.7 weeks); (B) Intermediate prognosis (score 2−3, median OS 37.3 weeks) and (C) Poor prognosis (score 4, median OS 13.4 weeks).
Conclusion: Pre-treatment CTC counts provide important prognostic data for the selection of patients for phase 1 clinical trials; prospective studies are now needed in unscreened patients to determine the clinical utility of CTC testing in this population.

604 POSTER

Farnesoid X receptor overexpression predicts breast cancer bone metastases through a Runx2-dependent mechanism

F. Journe[1], V. Durbecq[2], H. Boufker[1], D. Larsimont[2], G. Laurent[3], J.J. Body[4], A. Awada[5], G. Ghanem[1]. [1]*Institut Bordet, Lab Oncologie et Chirurgie Exp, Brussels, Belgium;* [2]*Institut Bordet, Département Pathologie, Brussels, Belgium;* [3]*Université de Mons, Service Histologie, Mons, Belgium;* [4]*CHU-Brugmann, Médecine Interne, Brussels, Belgium;* [5]*Institut Bordet, Oncologie Médicale, Brussels, Belgium*

Background: The skeleton is the most common site for breast cancer metastases. The bone matrix contains many growth factors, cytokines and lipids, which are released during osteolysis and may stimulate the proliferation of metastatic cells. Among lipids, bile acids have been recently reported to accumulate in bone tissue from serum and to promote the migration of human breast cancer cells. Bile acids act as physiological ligands for the farnesoid X receptor (FXR, NR1H4), a metabolic nuclear receptor endowed with ligand-dependent transcriptional activity. FXR is typically produced in the liver and the gastrointestinal tract, and we have demonstrated FXR protein expression in primary breast cancer.
Methods: We assessed FXR expression by IHC in primary breast tumors and correlated it with the site of metastasis. We also examined the possibility that FXR activation could induce the expression of bone-related factors in breast tumor cells.
Results: We found that FXR expression significantly correlated with the presence of bone metastases. Indeed, FXR was expressed in 98% of breast cancer samples ($n = 53$, median score = 6) of patients who developed bone metastases, while it was detected in only 68% of breast cancer specimens ($n = 28$, median score = 3) of patients with visceral metastases. Moreover, in the subgroup of patients with histological grade 3 tumors ($n = 18$), FXR was expressed at a high score (median = 7) in 100% breast cancer samples of patients who developed bone metastases, while it showed a much lower occurrence (50%) but also a lower score (median = 2) in patients who developed visceral metastases. Moreover, in the subgroup with high FXR expression, further analysis using a score cutoff at 5 gave a strong association between FXR and the development of bone metastases (positive predictive value of 91%). In parallel, we found that bile acids are able to stimulate the expression and binding to DNA of the transcription factor Runx2 as well as the extracellular structural protein osteopontin, at both the mRNA and protein levels, in the osteotropic MDA-MB-231 cells, but not in MCF-7 cells. The FXR antagonist guggulsterone significantly inhibited both effects.
Conclusions: Clinical and experimental data highly support a relationship between FXR overexpression in breast cancer and the propensity of the tumor cells to develop bone metastases, through a mechanism involving Runx2 stimulation and thus explaining the subsequent promotion of bone-related protein synthesis.

605 POSTER

Quantitative analyses of the impact of Akt inhibitor GDC-0068 on cell signaling and implications for clinical pharmacodynamic assessments

Y. Yan[1], V. Serra[2], D. Sampath[1], L. Prudkin[2], M. Scaltriti[2], B. Liederer[1], S. Murli[1], K. Lin[1], V. Ramakrishnan[1], J. Baselga[2]. [1]*Genentech, Research and Early Development (gRED), South San Francisco CA, USA;* [2]*Vall d'Hebron Institute of Oncology, Experimental Therapeutics Program and Pathology Department, Barcelona, Spain*

Background: Several classes of inhibitors of intracellular kinases are in preclinical and clinical development. Quantitative analyses of the effects of these inhibitors on signaling pathways would enable the use of mechanism-based biomarkers in clinical studies. Herein we describe the systematic analyses of serine and threonine phosphorylation events to generate a specific pharmacodynamic profile of GDC-0068, an inhibitor targeting the Akt node of the PI3 kinase pathway.
Methods: In order to dissect the impact of PI3 kinase pathway inhibitors on cell signaling, over 100 serine and threonine phosphorylation events were profiled using reverse-phase protein array (RPPA) at serial time points in five cell lines and three xenograft tumor models treated with varying concentrations of the Akt inhibitor GDC-0068 and rapamycin, an mTOR inhibitor. In addition, gene expression profiling and inhibitor concentration measurements in tumors were carried out in the tumor models.
Results: Phospho-protein profiling was used to demonstrate that the Akt inhibitor GDC-0068 suppressed tumor growth by down-regulating a selective set of phosphorylation events in the PI3K pathway, defining a profile corresponding to its main pharmacodynamic output. Interestingly, targeting Akt appeared to have distinct pharmacodynamic effects on signaling pathways *in vitro* and *in vivo* in tumor models that differed by PIK3CA, PTEN and HER2 status. Modeling the degree of signaling pathway inhibition in relation to tumor pharmacokinetics further refined the pharmacodynamic marker subset. Correlations with tumor growth measurements identified markers that correspond to tumor growth inhibition. The systematic analysis of signaling events revealed compensatory feedback loops that may relate to treatment escape mechanisms.
Conclusion: The quantitative pharmacodynamic analysis of protein phosphorylation in response to the Akt inhibitor GDC-0068 uncovered key signaling outputs that correlated with both tumor pharmacokinetics and tumor growth inhibition in preclinical models. This work defined mechanism-based biomarkers to assess the activity of the inhibitor in tumor biopsies of treated patients. In addition, this type of analysis has the potential to provide rationale to prioritize combination strategies to pursue in the clinic.

606 POSTER

Integrated analysis of genome-wide copy number and expression changes reveals novel genes in oesophageal adenocarcinoma

X.Y. Goh[1], J.R.E. Rees[1], A.L. Paterson[1], S.F. Chin[2], M. O'Donovan[3], D. Alderson[4], B. Ylstra[5], C. Caldas[2], R.C. Fitzgerald[1]. [1]*Hutchison-MRC Research Centre, MRC Cancer Cell Unit, Cambridge, United Kingdom;* [2]*Cambridge Research Institute, Cancer Research UK, Cambridge, United Kingdom;* [3]*Addenbrooke's Hospital NHS Trust, Department of Pathology, Cambridge, United Kingdom;* [4]*Queen Elizabeth Hospital, Academic Department of Surgery, Birmingham, United Kingdom;* [5]*VU University Medical Center, Department of Pathology, Amsterdam, The Netherlands*

Background: Genome-wide aberrations in oesophageal adenocarcinoma (OAC) are poorly characterised. We hypothesised that the discovery of putative important gene targets by integrating DNA and RNA profiles could enhance our understanding of the pathogenesis of OAC.
Method: Comparative genomic hybridisation (CGH) using an in-house 30K array was performed on 56 fresh frozen OAC resection samples from patients with long term-clinical follow-up. Common regions of aberrations (>5% samples) were called using swatCGH region detection algorithm. Matched gene expression microarray profiling data (median fold-change) was integrated with array CGH data ($\log_2$ ratios) to identify potential gene targets. Multiplex-nested PCR and quantitative fluorescence *in situ* hybridisation (qFISH) on tumour touch-imprints were used to confirm homozygous deletions (HDs). Immunohistochemistry (IHC) on OAC cores represented on tissue microarrays was used to validate targets with the most highly correlated copy number-expression changes on both internal ($n = 65$) and independent datasets ($n = 371$). Survival analyses were performed after unsupervised K-means clustering ($K = 5$, 50 iterations, reproducibility >50%) of array CGH data.
Results: 17 common regions of gains and 11 common regions of losses were identified. Integration of array CGH and expression data highlighted 6 potential gene targets (deletions of *p16* and *MBNL1*; gains of *EGFR*, *WT1*, *NEIL2*, *MTMR9*). Nested-multiplex PCR on microdissected tumour DNA and qFISH confirmed HD of tumour suppressor *p16*. IHC assays confirmed over-expression of EGFR (10% of tumours) and WT1 (20% of tumours), which was not restricted to tumours with gains. Survival analyses following clustering identified a group (32.1% of cohort) with significantly worse prognosis (median survival = 1.37 years; $p = 0.0149$). Modified T-test, with adjusted Bonferroni correction, identified 17 clones with different $\log_2$ ratios ($p < 4 \times 10^{-7}$), implicating 3 regions of gains (2p14, 7q22.1, 15q24.1) and including 5 novel genes (*ZMYND15*, *SYCP2L*, *PMP2*, *LYPD6* and *MEXD3*), between this group and all other groups combined.
Conclusion: Copy number gains with prognostic significance were identified using array CGH. Integration of array CGH and gene expression microarray data highlighted novel gene targets, including *WT1, NEIL2* and *MTMR9*. Overexpression of *EGFR* and *WT1* was observed in 10% and 20% of OAC respectively.Validation of novel genes *NEIL2* and *MTMR9* is currently underway. Functional validation will be required to determine the clinical relevance of these targets.