trifunctional antibody (AB) catumaxomab (anti-EpCAM x anti-CD3) has been approved for the i.p. treatment of patients with malignant ascites due to EpCAM+ carcinomas. By eradication of EpCAM+ tumour cells in the peritoneal cavity, catumaxomab effectively controls malignant ascites (MA). The aim of the reported investigation was to assess the frequency of EpCAM expression on tumour cells in malignant ascites.
Methods: The clinical phase IIIb study CASIMAS (NCT00822809, Fresenius Biotech) investigated a 3 hour i.p. infusion of catumaxomab with and without prednisolone premedication in MA patients.
Before treatment, ascites samples of 193 patients were collected for detection of EpCAM+ tumour cells. Cells were harvested, spun onto slides and labeled with the EpCAM-specific AB Ber-EP4. Cell-bound Ber-EP4 was detected with a biotinylated horse anti-mouse IgG and visualized with an avidin/biotinylated horseradish peroxidase complex and 3-amino-9-ethylcarbazole as substrate. Mayer's hemalaun was used as counterstaining. EpCAM+ cells were evaluated by light microscopy. The cytological data were related to the histology of the primary tumour.
Results: The main primary tumours of 193 patients evaluated were gastric, colon, pancreatic, breast, ovarian, lung and endometrial carcinomas.
In the majority of evaluable patient samples (n = 183, 95%) EpCAM+ tumour cells were detected (n = 170, 93%). In 2 patient samples, tumour cells were EpCAM-negative (1.1%) and in 11 patient samples tumour cells were not detectable (6.0%). 5.2% of the test samples were not evaluable. With regards to the primary tumour, in 100% of evaluable ascites samples from gastric, colon and endometrial, 98% from ovarian, 86% from breast, 80% from pancreatic and 85% of other carcinomas EpCAM+ tumour cells were detected.
Conclusions: The presented method for detection of EpCAM+ tumour cells in MA can be performed with standard laboratory equipment. In the ascites samples of the vast majority of patients, over all primary tumours investigated EpCAM+ tumour cells were detected at a high frequency. Based on these data it is concluded that the vast majority of patients with MA are indicated for an EpCAM targeted treatment with catumaxomab.

1095 POSTER

Growth Factor Receptor-targeted Therapy for Cancer

L. Ling[1], T.H. Goh[1], K.M. Foong[1], V. Nurcombe[1], S.M. Cool[1]. *[1]Institute of Medical Biology, Stem Cells and Tissue Repair Group, Singapore, Singapore*

Deregulated growth factor signaling aggressively stimulates the expression of oncogenes and is central to tumour growth and development. By selectively targeting the inhibition of FGF receptor (FGFR) signaling, our study is aimed at developing a potent treatment for cancer. To assess the sensitivity of cancer cells to FGFR-targeted therapy, we first screened the protein expression of FGFR by Western Blot analysis in seven lines of human osteosarcoma cells (including one primary osteosarcoma) and four lines of breast carcinoma cells, and compared their expression profiles with that of normal human osteoblasts and mammary gland epithelial cells. A neutralizing antibody against FGFR was developed and its effect on cancer cell growth and proliferation determined. Antibody specificity was evaluated by ELISA assays and the ability to neutralize FGFR activity examined using a Receptor Tyrosine Kinase (RTK) array. Lastly, the mechanism by which FGFR controls cancer cell growth and survival was determined by Western Blot analysis and Taqman Realtime PCR. Our data shows that FGFRs are upregulated in cancer cells, which prompted us to develop a novel antibody that significantly impairs FGF signaling and renders tumour cells unable to proliferate. This inhibition of cell growth is due to enhanced activation of several tumour suppressors, e.g. p53, Rb and FOXO3 as well as their target genes p21 and Bim. Our study demonstrates that FGF plays a key role in supporting cancer cell growth and that inactivating FGF signalling is a promising therapy to treat cancer. Future studies seek to determine whether the antibody is suitable for the treatment of cancer by examining its effect on animal tumour models.

1096 POSTER

Expression of Treg Associated Markers Have Prognostic Implications in Early-stage NSCLC

E. Jantus-Leintre[1], M. Usó[1], S. Gallach[1], E. Sanmartín[1], A. Blasco[2], R. Guijarro[3], C. Ortiz[4], M. Martorell[4], R. Sirera[5], C. Camps[2]. *[1]Fundación Investigación Hospital General Universitario de Valencia, Molecular Oncology, Valencia, [2]Hospital General Universitario de Valencia, Oncology, Valencia,[3]Hospital General Universitario de Valencia, Thoracic Surgery, Valencia, [4]Hospital General Universitario de Valencia, Pathologic Anatomy, Valencia, [5]Universidad Politécnica de Valencia, Biotecnology, Valencia, Spain*

Background: Tumour infiltration of lymphocytes is considered to be a manifestation of host immune reactions against cancers. CD4+, CD25high, CD127low and FOXP3+ regulatory T cells (Tregs) are believed to regulate T-cell immunity and to be the main obstacle in immunotherapy. A body of evidence suggests that Tregs within the tumour microenvironments might play a significant role in the suppression of local antitumour immune responses. The aim of this study was to determine the expression of these genes by RT-PCR and to correlate them with clinico-pathological and prognostic variables in resectable NSCLC.
Materials and Methods: RNA was isolated from 150 frozen lung specimens (tumour and normal lung) from untreated NSCLC patients (stages I-IIIA). RT-PCR was performed to analyze the expression of: CD4, CD25, CD127 and FOXP3 genes. Relative expression was normalized by an endogenous gene (GUSB) using the Pfaffl formulae. Statistical analyses were considered significant at $p < 0.05$.
Results: Tumour samples had significantly higher expression of CD25 (x2.1) and lower expression of CD127 (x0.42) than normal lung tissues, reflecting a Treg phenotype infiltrating the tumour. On univariate analysis, age (>65), PS and tumour size were associated with OS rates. Higher intratumoral levels of FOXP3 were associated with adverse prognosis ($p = 0.017$ for TTP and $p = 0.036$ for OS). On the other hand, the group of patients with higher CD4 tumour expression has increased TTP and OS. The combination of both variables (ratio FOXP3/CD4) allows the discrimination of a group of patient with poor prognosis, characterized by higher FOXP3/CD4 ratio ($p = 0.015$ for TTP and $p = 0.004$ for OS).
Conclusions: Our results show that the expression of Tregs markers seems to be associated with an increased risk of relapse and shorter OS. We also observed that the ratio FOXP3/CD4 can be used as prognostic marker in early-stage NSCLC patients (Supported by ISCIII PS09–01149 grant).

1097 POSTER

Correlation of Serum Biomarkers and Clinical Outcomes to Identify Predictors of Response to the Multi-targeted Kinase Inhibitor E7080 in Patients With Advanced Melanoma

D. Hong[1], J. Nemunaitis[2], Y. Funahashi[3], J.P. O'Brien[3], D. Verbel[3], T. Kadowaki[3], J. Simon[3], A. Eisen[3], X. Lucy[3]. *[1]The University of Texas MD Anderson Cancer Center, Department of Investigational Cancer Therapeutics, Houston, [2]Mary Crowley Medical Research Center, Executive Medical Director, Dallas, [3]Eisai Inc., Oncology, Woodcliff Lake, USA*

Background: E7080 is an oral TKI targeting VEGFR1−3, FGFR1−4, PDGFRβ, RET and KIT, which affects tumour cell proliferation and tumour vascularization in laboratory models. Tumour response (RECIST) and prolonged disease stabilization (>6 months) were observed in melanoma patients (pts) treated in phase I. The present study sought to identify biomarkers predictive of response to E7080 in melanoma.
Materials and Methods: 26 pts with metastatic melanoma received E7080 10 mg twice daily continuously on a phase I study. The pts responses were: 2 partial responses (PR), 14 stable disease (SD) >3 months, 1 SD >2 months, 5 progressive disease (PD) and 4 pts non evaluable. Serum was collected in these pts at baseline, 2hr after first dosing, C1D8, C1D15 and C1D22 for testing of five angiogenesis-related markers (PDGF-BB, soluble Tie-2, angiopoietin1, soluble e-selectin, soluble c-kit) and two apoptosis-related markers (cytochrome C and M30) by ELISA. The predictive value of lactate dehydrogenase (LDH) level, diastolic and systolic blood pressure (DIABP and SYSBP), hematocrit and hemoglobin at baseline and in the first cycle of treatment were also analyzed.
Results: The data were analyzed using Student's T-test based on clinical benefit (PR or SD $\geqslant$6 months); regression analysis for the continuous measure of tumour shrinkage and by Cox proportional regression based on progression free survival (PFS). The analysis identified baseline DIABP and SYSBP, PDGF-BB and angiopoietin1 level change at 2 hr after first dosing, change from baseline of soluble c-kit, M30, hematocrit and systolic BP at cycle 1 day 8 are associated with PFS by univariate analysis. By multivariate Cox Regression analysis, the ratio of PDGF-BB at 2 hrs to baseline (>1.15) and the baseline SYSBP (>132 mm/Hg) were significant predictors of PFS. The Kaplan–Meier curves illustrated that two parameter biomarkers (PDGF-BB 2hr ratio and baseline SYSBP) were better at stratifying low- and high-risk pts in terms of PFS than baseline LDH level for melanoma pts treated with E7080.
Conclusion: PDGF-BB 2hr ratio and baseline SYSBP appear to identify pts with advanced melanoma who may benefit from E7080 treatment. These predictive parameters will be integrated with on-going proteomic analysis of melanoma cells, gene expression data from tumour and vasculature cells and genotyping from these same tumours to model the response to E7080. The markers will be validated for their predictive value in an ongoing Ph 2 trial.