In the lung xenograft models, lapatinib was active towards NCI-H292 ($EGFR^{wt}$) as well as HCC-827 (delE746-A750) and demonstrated increased activity compared with cetuximab in this model as well as the non small cell lung K-Ras mutant line NCI-H441; however, lapatinib was only marginally active towards NCI-H1650 (delE746-A750) and inactive in NCI-H1975 (L858R/T790M). In addition, lapatinib was active towards the SK-OV-3 ovarian tumor model and demonstrated comparable activity to the multi TKI compounds sunitinib and sorafenib towards the EGFR and VEGF expressing A431 model. In these studies, no significant differences were noted in plasma: tumor ratios with comparable drug concentrations in both sensitive and insensitive tumors. In addition, some species differences were reported in half life and bioavailability studies.
Results from these studies benchmark lapatinib pharmacokinetic characteristics in rodents and humans and antitumor activity in various preclinical model systems which can be used to aid in the preclinical development of early stage anticancer agents.

70 POSTER

In vitro activity of the multi targeted receptor tyrosine kinase inhibitor sunitinib against multiple myeloma cell lines is not predictive of in vivo xenograft response

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The t(4;14) gene translocation in 15% of Multiple Myeloma (MM) patients leads to the ectopic expression of FGFR3 and is associated with a poor prognosis, suggesting that FGFR3 is a therapeutic target in this disease. In in vitro kinase assays, sunitinib has been identified as a potent FGFR3 inhibitor with an IC50 of between 120–300 nM. An interleukin 3 (IL-3) independent Baf/3 cell line engineered to express the ZNF198-FGFR1 fusion protein, and t(4;14) positive and negative MM cell lines, were used to screen for growth inhibition using the MTS assay. Sunitinib differentially inhibited the growth of Baf/3 cell transfectants compared to wild type cells, with a GI50 value of 730 nM versus 2.7 μM. Similarly, sunitinib induced differential growth inhibition of t(4:14) positive cell lines, with a GI50 of 1.2 μM compared to 4−5 μM for t(4:14) negative cell lines. No other FGFR isoforms were expressed in the MM cell lines. Sunitinib inhibited the activation of FGFR3 in t(4;14)-positive MM cells, and induced growth inhibition and apoptosis in a concentration and time-dependent manner in FGFR3-expressing MM cell lines. Consistent with growth inhibition, sunitinib was also shown to inhibit the activation of the MAPK pathway in MM cell lines through monitoring pERK1/2 levels by Western blot analysis. The in vivo efficacy of sunitinib was assessed using JIM1 (t(4:14) positive) and RPMI8226 (t(4:14) negative) subcutaneous xenografts grown in mice treated with vehicle control or 40 mg/kg sunitinib daily by oral administration for 21 days. In contrast to the in vitro data, treatment with sunitinib gave only a minor 2 day growth delay in the t(4:14) positive JIM1 xenograft model, whereas marked antitumour activity was observed in the t(4:14) negative RPMI8226 model with at least a 15 day growth delay. These results suggest that the in vivo efficacy of sunitinib is not solely due to cellular determinants, but is also dependent on other factors such as, potentially, host stroma interactions. Furthermore, these studies caution against using the t(4:14) translocation as a predictive biomarker for sunitinb sensitivity in MM.

71 POSTER

Features of chemo and radiotherapy response of a new model of breast cancer xenograft derived from a BRCA2 germ-line mutation carrier patient's tumour

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Background: BRCA germline mutations predispose to breast and ovarian cancers that have high propensity to relapse rapidly after response to the initial treatment. Several clinical agents exert their cytotoxic effect through the formation of double-strand DNA breaks and the effect of BRCA2 on DNA repair might be exploited clinically. The purpose of this work is to characterize a new xenografts with BRCA2 gene mutation that can be useful for the study of the biology of BRCA2-deficient breast cancers and to test new drugs.
Patient's tumor, material and methods: A BRCA2-deficient breast cancer xenograft was established directly from a patient's tumor, of a 37-year old woman. The patient had a BRCA2 germ-line mutation and was treated by mastectomy, taxane-based chemotherapy and parietal radiotherapy. The clinical control of the disease was achieved with a cisplatinum-ifosfamide combination. Characterization of the HBCx-17 xenograft was performed and compared to that of patient's tumor, including histological analysis, BRCA2 gene sequencing, p53 status determination and genetic analysis by comparative genomic hybridization (CGHarray). Detection of gamma-H2AX repair foci was done by immunofluorescence microscopy. Sensitivity to different standard chemotherapies and radiosensitivity of the HBCx-17 xenograft were evaluated. A cell line was also obtained from the xenograft.
Results: The xenograft presented the same features than the primary tumor. HBC-17 was identified as a triple negative breast cancer (ER−, PR−, ERBB2−) with a high EGFR expression and a mutated p53. Ki67 staining revealed a high proliferation status in both patient and xenograft cancer cells and CGHarray showed a high number of gene alterations. Therapeutic assessment of the xenograft showed sensitivity to anthracyclin-based chemotherapy, to radiotherapy and cisplatin-based treatments and lack of sensitivity to taxane. Deficiency in DNA DSB repair was found by staining the phosphorylated γ-H2AX foci after γ-irradiation. Loss of heterozygosity at the BRCA2 locus was detected by poly-CA analysis.
Conclusions: This study describes the characterization of a new human breast cancer xenograft model obtained directly from a BRCA2 gene mutated patient. This tumor xenograft represents a unique *in vivo* model of triple negative BRCA2 mutated breast cancer conserving the same biological caracteristics of the patient tumor, providing a useful tool for testing new agents and protocols and for further exploration of the biological basis of drug responses.

72 POSTER

Preclinical evaluation of the tyrosine kinase inhibitor erlotinib: Bioanalytical method development and pharmacokinetic analysis and in vivo evaluation and comparison in a panel of human EGFR wildtype and mutant tumor xenograft models

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The epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor erlotinib is currently used for treatment of non-small cell lung cancer and as part of a combination therapy in some pancreatic cancers. Because of its mechanism of action and positive preclinical results, clinical trials are underway examining the possibility of expanding the use of this agent in other indications both alone and in combination with approved chemotherapeutics or novel agents in later stages of development. Several early-stage compounds have shown promising single agent activity in preclinical studies and may be useful when combined with erlotinib in treatment of certain cancer types.
Our goal was to benchmark erlotinib in various preclinical systems for subsequent studies evaluating effects of early-stage anticancer agents on erlotinib pharmacokinetics, tumor and tissue deposition, and antitumor activity. To accomplish this, we developed a bioanalytical method for quantification of erlotinib in mouse and rat plasma and tissue; experimental determinations included half-life with single and repeated dosing, oral versus intravenous bioavailability, and determination of plasma: tumor and tissue ratios. In addition, we screened a panel of human tumor xenograft models including non-small cell lung based on EGFR mutation status, practical tissue types, and published results. Activity of erlotinib was also compared to the EGFR-targeting antibody cetuximab in several of these models.
In the lung xenograft models, erlotinib was active towards NCI-H292 ($EGFR^{wt}$) as well as HCC-827 and NCI-H1650 (delE746-A750) and demonstrated increased activity compared with cetuximab in both 746–750 deletion mutants as well as the non small cell lung line NCI-H441 ($K\text{-}Ras^{mut}$); however, erlotinib was inactive towards the NCI-H1975 (L858R/T790M) model. No significant differences were noted in plasma: tumor ratios with comparable drug concentrations in both sensitive and insensitive tumors. In addition, some species differences were reported in half life and bioavailability studies.
Results from these studies benchmark erlotinib pharmacokinetic characteristics in rodents and antitumor activity in various model systems which can be used to aid in the preclinical development of early stage anticancer agents.