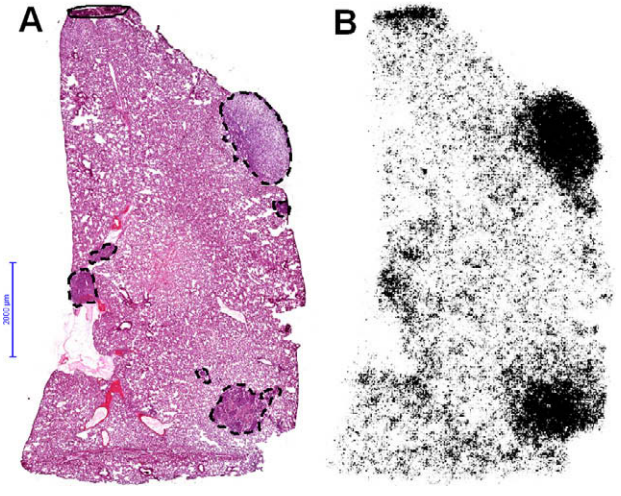
lung metastases. On day 26, the primary tumor was surgically removed. The radiosynthesis of D-FMT was carried out using an indirect labeling method. Micro-PET imaging was performed before (22 days after tumor cell inoculation) and after removal of the primary tumor (day 28/29). After micro-PET imaging samples of tumor, scar and lung tissues were collected for autoradiography and histological studies.
Results: Both tracers, D-FMT and FDG allowed good visualization of primary 4T1 tumors in vivo. In contrast to D-FMT, FDG also showed uptake in additional organs leading to high background signal. Due to high background particularly in the heart, FDG was not able to detect lung metastases whereas D-FMT could light up all lesions. Autoradiography of lung tissue after D-FMT imaging confirmed the specific uptake of D-FMT into the metastases but not in normal lung tissue. FDG-PET imaging revealed additional sites of uptake in the area where the primary tumor was removed. Histological examination confirmed the presence of significant amounts of macrophages most likely responsible for FDG uptake into inflamed areas. In contrast, no uptake of D-FMT into inflamed tissue was observed.
Conclusion: These data qualify [F-18]-D-FMT as a new potential imaging tracer for the *in vivo* detection of primary tumors as well as metastases with high specificity to reliably differentiate tumor lesions from inflamed tissue. Therefore, this new tracer should be useful not only for improved diagnosis but also for therapy control, especially in course of external beam radiation.



Histological section with H&E stain (A) and autoradiography from in vivo D-FMT imaging (B) of a lung from the 4T1 metastasis model showing increased radioactive signal in the metastases. Metastases are delineated by dashed black line. The artefact from tissue folding is marked by a solid black line.

595 POSTER

Preclinical activity and the role of biomarkers of sensitivity to the selective phosphatidylinositol 3′kinase inhibitor GDC-0941 in breast cancer

M. Lackner[1], C. O'Brien[1], J. Fridlyand[1], A. Pandita[1], S. Boyd[1], E. Punnoose[1], H. Koeppen[2], L. Friedman[3], L. Amler[1], G. Hampton[1].
[1]Genentech Inc., Oncology Diagnostics, South San Francisco CA, USA; [2]Genentech Inc., Pathology, South San Francisco CA, USA; [3]Genentech Inc., Cancer Signaling, South San Francisco CA, USA

Background: The class I phosphatidylinositol 3′kinase (PI3K) is activated in a wide variety of human malignancies and inhibitors targeting the PI3K pathway hold great promise in the treatment and management of cancer. Successful development of such inhibitors will be enhanced by identification of responsive patients through the use of predictive biomarkers.
Materials and Methods: We used cell lines and xenograft tumor models with accompanying molecular and genetic characterization to evaluate a collection of putative biomarkers predictive of response to the selective inhibitor GDC-0941 in breast cancer.
Results: We found excellent activity of the PI3K inhibitor GDC-0941 in luminal and HER2 amplified models, and that PIK3CA mutations and HER2 amplification are highly specific biomarkers of response to this agent. PTEN was predictive in some but not all cases, suggesting a need for further biomarkers of response in PTEN-driven disease.
Analysis of human breast tumor samples, including matched primary tumor and lymph node metastases from 59 patients, suggested that primary and metastatic sites were generally concordant in terms of status of key biomarkers such as PTEN and PIK3CA, though several notable exceptions were discovered. We also compared PTEN status determined by immunohistochemistry (IHC) with a chromosomal fluorescence in situ hybridization (FISH) assay in a subset of tumors and found evidence for chromosomal deletion of the PTEN locus in several ER+ breast cancers, suggesting that some but not all of the PTEN loss observed in breast occurs at the chromosomal level and that both FISH and IHC may have utility as diagnostic assays.
The high prevalence of pathway activating events in ER+ breast cancer, along with strong single agent GDC-0941 activity, suggest it is an attractive indication for development of PI3K inhibitors. Based on this, we explored the rational combination of the pure estrogen receptor antagonist Fulvestrant with GDC-0941 and will report on these results.
Finally, we found that a number of breast cancer models that do not harbor known pathway alterations also showed sensitivity to GDC-0941, suggesting a need for additional diagnostic markers. Gene expression studies identified a collection of genes whose expression was associated with in vitro sensitivity to GDC-0941, and expression of a subset of these genes was found to be intimately linked to signaling through the pathway. We will discuss the prognostic significance of this gene signature and relationship with other clinicopathologic parameters.
Conclusions: Our data provide a strong rationale for developing a selective PI3K inhibitor in the ER+ breast cancer setting, and a framework for clinical implementation of predictive biomarker assays that may have utility in patient selection.

596 POSTER

Design and validation of pharmacodynamic assays to measure the activity of the HSP90 inhibitor, AT13387 in surrogate tissue and tumor in a phase I study

J. Lyons[1], M. Squires[1], V. Lock[1], B. Graham[1], T. Smyth[1], E. Ong[2], D. Mahadevan[2], E. Kwak[3], G. Shapiro[3]. *[1]Astex Therapeutics Limited, Translational Research, Cambridge, United Kingdom; [2]Arizona Cancer Center, Surgery, Tucson, USA; [3]Dana Farber Cancer Institute, Oncology, Boston, USA*

Heat Shock Protein 90 (HSP90) is a member of a family of molecular chaperone proteins which directs the folding of polypeptides into functional configurations affecting stabilisation and activation. AT13387 is a small molecule inhibitor of HSP90 discovered using fragment-based drug discovery. Pharmacokinetic studies in tumor bearing mice showed that AT13387 exhibits a much extended tumor half life compared to that in plasma.
The studies presented here characterise the kinetics of pharmacodynamic (PD) activity in mouse models and how they may correlate with efficacy on a particular dose schedule. These data were then used to validate and translate a suite of laboratory assays into a biomarker platform for use on clinical samples. Plasma and tumour samples from a phase I clinical study were used to develop and confirm a set of PD biomarker assays to assess the level of HSP90 inhibition in patient samples.
We show here that a xenograft tumor half life of up to 72 hours results in the modulation of markers of HSP90 inhibition; including an induction of HSP70 and a reduction in the levels of client proteins for between 6 and 96h. This extended PD effect predicted efficacy on both once or twice weekly dose schedules and this was confirmed in a number of xenograft models. An HSP70 ELISA assay in peripheral blood mononuclear cells (PBMCs) was developed and again, in the mouse model, HSP70 induction was observed at between 1 and 6h, consistent with the plasma half life of AT13387 at 4 hours. There was a dose dependent effect of AT13387 on HSP70 induction resulting in a significant increase at doses above 60 mg/kg. We confirmed that the HSP70 ELISA effectively monitored HSP70 in human PBMCs in an *ex vivo* assay and used the dose and time dependency data to design a sampling procedure for the phase I clinical study.
PD data generated during a phase I study with AT13387 in refractory solid malignancies confirmed pre-clinical observations of the dose and time dependency of HSP70 induction in patients PBMCs along with some examples of client protein knockdown. We conclude that we achieve sufficient plasma levels to inhibit HSP90 in PBMCs in all cohorts in this study. This level of inhibition results in client protein degradation in several instances. We go on to demonstrate in 5 paired tumor biopsies, taken in the MTD cohort, that we achieve pharmacologically active concentrations of AT13387 in the tumor as demonstrated by HSP70 induction, modulation of client proteins and markers of apoptosis. These data represent a case study in translating assays applied to pre-clinical models to clinical biomarker assays with the aim of demonstrating pharmacological activity of AT13387 in clinical samples and informing the minimally effective biological dose on a twice weekly dose schedule.