p27, p53, MDM2, E2F1 and, Androgen receptor. Du145+Id4 cells have significantly decreased proliferation due to an S-phase arrest suggested by an E2F-1 intermediary response. The increased expression of E-cadherin, p27, p21 and p53 strengthens the hypothesis of Id4 as a tumor suppressor by regulating key cell cycle control and apoptosis associated genes. A functional androgen receptor marked a dramatic change in the androgen independent prostate cancer cell type. Id4 ectopic expression resulted in a significant decrease in Id1 and Id3, which are known contributors to metastasis and cell survival. Id4's role may be to inhibit bHLH transcription factors involved in proliferation, metastasis, apoptosis and senescence. Id4 induces apoptosis whether dependent/independent of the mutated p53 gene in DU145 cells. The presence of senescent cells in Id4 transfected cell lines suggest that Id4 may also play a role in autophagic cell death. As a transcription factor Id4 has the capability of inducing a total cellular reprogramming, by influencing a number of key cellular pathways. We conclude that the tumor suppressor function of Id4 is responsible for causing a reversal in the cancer phenotype of the cell by inducing apoptosis, senescence, S-phase mediated cell cycle arrest resulting in a molecular and morphological change.

591 POSTER

Berberine sensitizes TRAIL-induced apoptosis through proteasome-mediated down-regulation of c-FLIP and Mcl-1 proteins

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Berberine is an isoquinoline alkaloid used in traditional Chinese medicine and has been isolated from a variety of plants, such as *Coptis chinensis* and *Phellodendron amurense*. It has a wide spectrum of clinical applications such as in anti-tumor, anti-microbial, and anti-inflammatory activities. We showed that co-treatment with subtoxic doses of berberine and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) induced apoptosis in human renal cancer cells, Caki cells, but not in normal tubular kidney cells. Treatment of Caki cells with berberine down-regulated c-FLIP and Mcl-1 proteins in dose- and time-dependent manners. Interestingly, berberine-induced decreases in c-FLIP and MCl-1 protein levels were involved in proteasome dependent pathways, which was confirmed by the result that pretreatment with proteasome inhibitor, MG132, inhibited berberine-induced down-regulations of both c-FLIP and Mcl-1 proteins. Pretreatment with N-acetyl-l-cysteine (NAC) significantly inhibited the cell death induced by the combined treatment with berberine and TRAIL as well as recovered the expression levels of c-FLIP and Mcl-1 down-regulated by the combinatory treatment with berberine plus TRAIL, suggesting that berberine-stimulated TRAIL-induced apoptosis appears to be dependent on the generation of reactive oxygen species for down-regulation of c-FLIP and Mcl-1. Taken together, the present study demonstrates that berberine enhances TRAIL-induced apoptosis in human renal cancer cells by ROS-mediated c-FLIP and Mcl-1 down-regulations.

592 POSTER

Characterisation of novel, small molecule antagonists of XIAP, cIAP1 and cIAP2 generated by fragment based drug discovery (FBDD)

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The inhibitor of apoptosis (IAP) family of proteins are important regulators of cancer cell survival, making them attractive targets for cancer therapy. They are characterized by one to three baculovirus IAP repeat (BIR) domains, which are necessary for the antiapoptotic activity of most IAPs. Several small molecule BIR antagonists mimic the N-terminal sequence of SMAC (second mitochondrial activator of caspases), an endogenous inhibitor of the IAPs. These peptidomimetic compounds have the ability to sensitise and/or promote apoptosis in cancer cells and inhibit tumor growth *in vivo*. Using our fragment-based screening approach, Pyramid™, we identified a range of diverse, non-peptidomimetic chemotypes which bind to the P1′−P2′ pocket in the BIR3 domain of XIAP. Alanine-like fragments have also been identified with excellent Ligand Efficiency (LE) values, which are superior to LE of Ala-Val (natural substrate) and to LEs of published competitor compounds. Optimisation of these hits using a structure based approach led to novel series (both alanine and non-alanine) which bound with sub μM potency to both XIAP and cIAP1. The most potent compounds were characterised further in proliferation assays using two sensitive human breast cancer cell lines EVSA-T and MDA-MB-231 (with an insensitive cell line, HCT116, as a control). Anti-proliferative compounds were investigated further for their ability to induce cIAP1 degradation and to increase the levels of cleaved caspase-3 in EVSA-T cells. cIAP1 degradation occurred rapidly at low compound concentrations in all cell lines tested; whilst caspase-3 induction closely paralleled the anti-proliferative data. In conclusion fragment-based screening has enabled the identification of non-peptidomimetic ligands that inhibit this protein:protein interaction. These chemotypes represent promising start points for novel, selective IAP antagonists.

593 POSTER

Notch-1 fragment peptide induces autophagy and caspase-independent cell death in leukemic cell lines

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Background: Non-apoptotic cell death such as autophagic cell death or necrosis is an important physiological process. Potent inducers of non-apoptotic cell death promise to be a valuable tool for development of novel chemotherapeutic agents. In this study, we found that a 25mer peptide conjugate induces a caspase-independent cell death in multiple human leukemic cell lines.
Material and Methods: All the peptides were synthesized by using an Fmoc chemistry-based automatic peptide synthesizer. Human monocytic cells and leukemic cell lines were maintained in RPMI-1640 medium supplemented with 10% fetal calf serum. Measurements of cell viability and mitochondrial membrane potential were determined by WST-8 chromogenic assay and fluorescence-activated cell sorter using the JC-1 dye, respectively.
Results: To suppress Notch signaling which is overactivated in T-cell leukemia and glioma, we designed Tat-Notch-1 fragment peptide (Tat-NF), which is a cell penetrating HIV-1 tat-conjugate with a 13mer peptide fragment (R-R-Q-H-G-Q-L-W-F-P-E-G-F) derived from Notch-1 intracellular domain. Unfortunately, the conjugate has no inhibitory effect on the Notch signaling in malignant gliomas, whereas the gamma-secretase inhibitor blocked the signal. However, the Tat-NF rapidly killed almost the leukemic cells (Jurkat-T, CCRF-CEM, Molt-4 etc.) tested in a tumor cell-specific manner. Single alanine substitutions of the LWF motif caused a significant decrease of the peptide-inducing cell death. Pharmacological inhibition of caspase activity did not prevent the cell death, although mitochondrial membrane potential was significantly decreased. In the cells undergoing such cell death, we observed the conversion of the soluble LC3-I to the autophagic vesicle-associated LC3-II and the formation of lysosomes/autophagosomes.
Conclusions: The 13mer peptide conjugated with HIV-1 tat has an ability to induce autophagy and caspase-independent cell death without affecting the Notch signaling. These data suggest that the conjugate is useful to elucidate the molecular mechanism of non-apoptotic cell death and to develop novel chemotherapeutic agents for treating leukemia with apoptotic defects.

Biomarkers

594 POSTER

In vivo detection of mammary tumor and its lung metastases in the 4T1 metastasis mouse model by PET imaging using [F-18]-D-FMT (BAY 869596)

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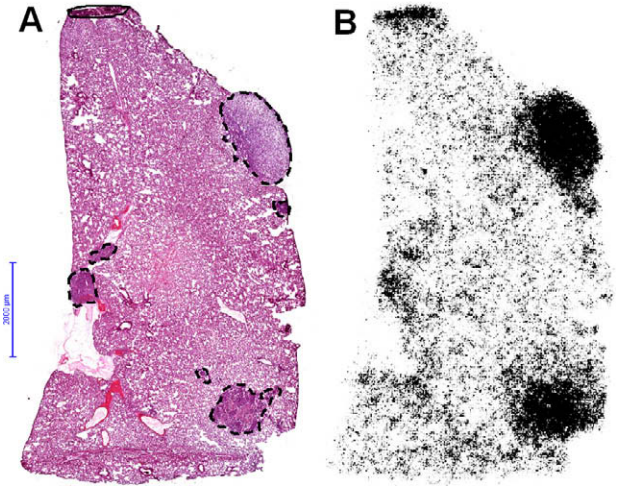
Background: Detection and localization of metastatic lesions and their differentiation from therapy induced inflammation is still a difficult obstacle for diagnosis and subsequent treatment decisions. Positron emission tomography (PET) allows the sensitive detection of radioactive labeled molecules, which specifically accumulate in tumor tissue. This offers a promising tool to assess molecular details about the disease comprehensively and contributes to an optimized cancer patient management and therapy control. At present [F-18]-fluorodeoxyglucose (FDG) is the most frequently used PET tracer in oncology. However, FDG has limited specificity by accumulating also in inflammatory cells due to their increased glucose metabolism. To overcome this problem, amino acids have been investigated. D-[F-18]-fluoromethyl tyrosine (D-FMT, (R)-2-amino-3-(4-[F-18]fluoromethoxy-phenyl)-propionic acid) has shown good uptake into HeLa tumors in nude mice with no accumulation in sterile induced inflammation sites. The aim of this study was to investigate D-FMT in a metastasis model for its capability to detect the primary tumor as well as metastatic lesions and differentiate metastatic from inflammatory sites.
Material and Methods: 2.5×10^5 4T1 mouse mammary carcinoma cells were implanted subcutaneously in NMRI mice, which generated several

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

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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

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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.