multiple molecular events contribute to the apoptotic effect of 5-FU, by which E2F-1 activation and MEK inactivation coordinate with p53 generated signals to induce efficient apoptosis.

635 POSTER

Inflammatory response might influence the pharmacokinetics (PK) and pharmacodynamics (PD) of Imatinib and CGP 74588 in patients with advanced gastro-intestinal-sarcoma (GIST)

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Background: Ten percent of patients with advanced GIST presented primary resistance and 20% developed secondary resistance per year of Imatinib (Gleevec) treatment. Possible mechanism for resistance might be low drug exposure. Prior PK analyses of Imatinib showed large inter-individual variability in patients. This study was designed to explore the factors affecting PK variability of Imatinib and its main metabolite, CGP 74588, along with PK-PD correlations.
Methods: Thirty-five patients (26 males; median age 55 yrs, range 28–84 yrs) with advanced GIST, registered in the French Sarcoma Group phase III study (BFR14 trial), received 400 mg/d of Imatinib. Five blood samples were obtained before intake, between 1 and 3 hours and 6 and 9 hours on day 1, prior to next dose on day 2 and at steady state on days 30 and 60. Imatinib and CGP 74588 plasma levels were quantitated by reverse-phase HPLC coupled with tandem mass spectometry, and analysed by population PK using NONMEM program. We examined the influence of 17 covariates on Imatinib clearance (CL) and apparent CGP 74 588 clearance (CLM/fm, with fm = fraction of Imatinib converted to CGP 74588). These covariates included age, weight, gender, alpha-1-acid glycoprotein (AAG), renal, hematological and liver biological values at baseline along with oedema, liver metastasis and occasion (OCC = 0 if PK data obtained at day 1, or = 1 at day * 30).
Results: Both clearances (CL and CLM) decreased in case of elevated AAG, probably due to higher plasma protein binding with a best regression formulas of: CL = 17.2/(1 + 0.961*AAG), and CLM/fm = 164*(1 − 0.46*OCC)/(1 + 1.52*AAG) (AAG in g/L). A significant time-dependent decrease in CLM/fm was evidenced with a mean+SD CGP/Imatinib AUC ratio of 0.25+0.07 at steady state, compared to 0.14+0.03 on day 1. Hematological toxicity, measured by the relative decrease in absolute neutrophil count (ANC) [ΔANC = (ANC nadir − ANC on day 1)/ANC on day 1) and in Δ platelets, was significantly correlated with high exposure to Imatinib on day 1 and at steady state, particularly if considering unbound plasma Imatinib concentration at steady state. Significant correlation between ΔANC and AAG was observed on day 1 ($p<0.0001$). Response and oedema occurrence were not correlated with any PK parameters.
Conclusion: Inflammatory response might influence the metabolism, the drug disposition and the hematological toxicity of Imatinib in with advanced GIST.

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Promoter hypermethylation of the DNA repair gene mgmt is more frequent in secondary glioblastomas and is independent from other prognostic factors

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O^6-methylguanine-DNA methyltransferase (MGMT) is a DNA repair protein that specifically removes promutagenic alkyl groups from the O^6 position of guanine in DNA. Repair of cytotoxic DNA damage by MGMT is a potentially important factor of resistance to alkylating chemotherapeutic agents, commonly used in the treatment of glioblastoma multiforme (GBM) since it reduces the cytotoxicity of these drugs.
We assessed the inactivation of the DNA-repair gene MGMT by promoter hypermethylation using Methylation-Specific PCR (MSP) in 45 GBM obtained from patients subsequently treated by conventional radiotherapy and CDDP+BCNU. We observed that the MGMT gene was methylated in 15 patients (33%). This finding was associated with prolonged overall survival (25 versus 14 months; log-rank p=0.026) and with a longer Progression Free Survival (PFS) (11 versus 7 months; log-rank p=0.037). Secondary GBMs had prolonged overall survival (30 versus 11 months; log-rank p=0.0030) than *de novo* tumors, whereas other prognostic factors were not statistically associated with ST or PFS. Moreover, methylation status was more frequent in secondary than in primary GBMs (70% versus 23%, p=0.0091), but was not associated with other clinical parameters. Other genetic markers as EGFR amplification, p53 mutations and microsatellites analysis for loss of heterozigosity are under study to assess their influence on the treatment response and overall survival of patients with GBM.

637 POSTER

Effective combinations of carboplatin with low doses of TRAIL, HGS-ETR1 and HGS-ETR2 in the TRAIL-sensitive HX62 human ovarian tumour cell line

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TNF-related apoptosis inducing ligand (TRAIL) has the ability to induce apoptosis in cancer cells, with minimal toxicity in normal cells, in pre-clinical models, via the extrinsic pathway. A strategy that may overcome drug resistance in ovarian cancer is to combine cytotoxic agents with TRAIL, engendering a partnership between the extrinsic and intrinsic pathways of apoptosis. Ovarian cancer cell lines are often resistant to TRAIL, as we have observed in 5 of our panel of 7 lines (IC50s >3 μg/ml). The expression levels of receptors DR4, DR5 and DcR2, caspase-8 and XIAP are similar in these lines. We evaluated the effect of combining TRAIL with carboplatin in the TRAIL sensitive, carboplatin resistant cell line, HX62 (TRAIL IC50 = 387±117ng/ml) using an MTT growth inhibition assay as the endpoint and analysed using the Median Effect equation as described by Chou and Talalay. An ~IC25 dose of TRAIL (150ng/ml) was shown to be additive (CI_{ED50} value = 1.0) when combined with increasing doses of carboplatin (CI_{ED50} = 1.1±0.16). We performed similar studies with the agonistic antibodies to TRAIL receptors, HGS-ETR1 and HGS-ETR2. HX62 cells were sensitive to both agents (IC50 = 0.16 and 1.8 μg/ml respectively). HGS-ETR1, at 0.05 μg/ml (minimally growth inhibitory) sensitised HX62 cells to carboplatin, shifting the IC50 from 60±13 μM to 24±13 μM (p= 0.029) and the data suggest this is a synergistic interaction (CI_{ED50}<1.0); CI_{ED50} = 0.85±0.31. Reducing the HGS-ETR1 dose to 0.01 μg/ml (~6% of IC50; non-growth inhibitory) also resulted in sensitisation (CI_{ED50} = 0.79; n=2). A non-growth inhibitory dose of HGS-ETR2 (0.1 μg/ml; ~5% of IC50) induced similar sensitisation; CI_{ED50}= 0.75; n=2. SKOV-3 cells, in comparison, are resistant to TRAIL (no growth inhibition at 3 μg/ml) and also resistant to HGS-ETR1 and HGS-ETR2 (no inhibition at 10 μg/ml). Preliminary studies show no sensitisation when TRAIL (500ng/ml) was combined with carboplatin. This work will be extended to other cell lines and the reasons for TRAIL resistance in cell lines such as SKOV-3 will be investigated. In conclusion, the agonistic antibodies, HGS-ETR1 and HGS-ETR2 are effective alone and in combination with carboplatin in a TRAIL-sensitive ovarian tumour cell line.
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638 POSTER

Acquired resistance to EGF receptor-targeted cancer therapy

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Pharmacological approaches to target the epidermal growth factor receptor (EGFR) in cancer cells include monoclonal antibodies that block ligand binding to the receptor and small-molecule inhibitors that compete for the ATP binding site on the receptor. Two leading agents, Iressa (gefitinib/ZD1839) and Erbitux (cetuximab/C225) were recently approved by the US Food and Drug Administration for the treatment of patients with chemorefractory lung cancers and colon cancers, respectively. With the incorporation of this novel anti-cancer therapy into standard practice, it is anticipated that acquired resistance to the treatment may occur. The purpose of this study is to develop experimental models to explore potential molecular changes associated with the acquired resistance. We developed two types of resistant sublines from the DiFi colon cancer cells, which have an innate sensitivity to EGFR inhibition, by exposing the cells to sub-effective doses of C225 (DiFi5 cells) or AG1478 (DiFi-AG cells) for extended time periods. Compared with parental DiFi cells (DiFi-P), DiFi5 cells exhibit remarkable reduction in the level of EGFR (approximately equal to 10% of the EGFR in DiFi-P cells) and slight reduction in growth rate, and become insensitive to C225 or AG1478. In contrast, DiFi-AG cells showed similar level of EGFR and slightly increased growth rate, and are resistant

to AG1478 but still very sensitive to C225. Other remarkable differences among DiFi-P, DiFi5 and DiFi-AG cells are the basal and EGF-stimulated phosphorylation levels of MAPK and Akt. The MAPK is constitutively activated (phosphorylated) and is insensitive to EGF stimulation in DiFi5 cells. In contrast, the basal levels of phosphorylated MAPK are low, and can be stimulated by EGF in DiFi-P and DiFi-AG cells. The basal levels of Akt phosphorylation are low in DiFi-P and the two sublines, and can be stimulated by EGF in DiFi-P and DiFi-5 cells, but not in DiFi-AG cells. Expression profile analysis with the Affymetrix microarray chips (U133A) showed that DiFi-P is clustered in the same group with DiFi-AG, however, principal component analysis (PCA) result shows that DiFi-P is distinct from DiFi-5 and DiFi-AG cells in the component-2 direction. There are 299 genes differentially expressed between DiFi-P and the two DiFi-resistant variants. We are currently validating and screening these differentially expressed genes using the "training and test" approaches, which may contribute to the acquired resistance.

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Characterization of the binding sites of P-glycoprotein by a functional flow cytometric assay

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Background: The overexpression of the MDR1 product P-glycoprotein (P-gp) is often responsible for limiting the success of cancer chemotherapy. P-gp is known to bind to and transport a wide variety of agents. Different drug binding sites have been proposed. Daunomycin and Hoechst 33342 have been shown to bind to different sites, which interact in a positively cooperative manner. We developed a functional flow cytometric assay searching not only for new modulators but focusing on the characterization of their binding sites as a basis for molecular modeling analysis aiming to understand the mode of action of P-gp.
Material and methods: P-gp activity was measured using a flow cytometry assay based on daunomycin influx. Measurement was gated to include only single, viable cells. Concentration-dependent effects of the P-gp modulators verapamil, imatinib, Hoechst 33342 and quercetin on daunomycin influx were determined in the P-gp expressing cell line A2780adr. Controls were incubated without modulator.
Results: Incubation of A2780adr with Hoechst 33342 stimulated P-gp activity and led to a decrease in daunomycin influx, whereas verapamil and imatinib inhibited P-gp activity significantly. Quercetin showed a biphasic effect. Lower concentrations of Quercetin decreased, concentrations above 10^{-6}M increased daunomycin influx, respectively.
Conclusions: Modulators interacting with the Hoechst binding site stimulate the daunomycin binding site in a positively cooperative manner and decrease daunomycin influx, whereas modulators of the daunomycin binding site, like e.g. verapamil and imatinib, increase daunomycin influx. The preliminary results of our study show that the developed assay is well suited for the characterization of the P-gp binding sites. Our data correlate well with the binding sites proposed in literature. The benefit of our method is the in situ measurement of P-gp activity using intact cells instead of membrane vesicles with reconstituted protein.

Radiation interactive agents

640 POSTER

Stat1 as mediator of acquired tumor radioresistance and potential target for anti-tumor therapy

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Background. Mechanisms of acquired tumor radioresistance are an area of intense study. To approach understanding of these mechanisms we selected in vivo radioresistant tumors nu61 from the radiosensitive SCC-61. Expressional profiling revealed that nu61 tumors constitutively overexpressed sets of IFN-inducible genes and Stat1 compared with radiosensitive SCC61. We proposed that overexpression of the Stat1 may be critical for the radioresistance (Khodarev et al., PNAS, 2004, 101:1714). In the current report we investigated the effects of ionizing radiation on Stat1 expression in different cell lines and effects of Stat1 overexpression on clonogenic survival of transfected clones.
Materials and Methods. Selection of stably transfected clones and Western analysis are described in (Khodarev et al., PNAS, 2004, 101:1714). In the current report we used only β-1, α-16 and MT-4 clones. Clonogenic analysis was performed in the dose range between 0 and 10Gy. siRNA was synthesized with Silencer™ kit (Ambion, USA) and IFN measurements performed with R&D kits (R&D Systems, USA).
Results. Fractionated IR (3×5Gy) led to the up-regulation of Stat1 protein in 9 cell lines from breast, prostate, colon and head and neck cancer. Up-regulation varied from 1.2- to 5.1-fold at 24 hours after last dose. After single dose (5Gy) Stat1 up-regulation was detected at 30 min and reached a plateau at 8 hours. IR-induced up-regulation of Stat1 precedes the IR-induced production of IFNα. Clonogenic assays of β-1, α-16 and MT-4 clones revealed that β-1 and α-16 were significantly more radioresistent than mock-transfected clone MT-4 (6.9-fold and 9.7-fold respectively at 10Gy). Also, constitutive overexpression of Stat1 in β-1 clone led to the overexpression of IFN-inducible genes, previously detected in nu61 in vivo. Anti-Stat1 siRNA led to the 4.5-fold suppression of the cell growth of the radioresistent tumor cell line nu61.
Conclusions. 1. Ionizing radiation leads to the up-regulation of Stat1 common in tumor cell lines surveyed. 2. Stat1 mediates radioresistance and induction of IFN-inducible genes, which recapitulates radioresistant phenotype of nu61 tumor. Consistently suppression of Stat1 leads to the growth suppression of nu61. 3. IR-induced up-regulation of Stat1 is an early IFN-independent event. Data suggest that Stat1 is an important mediator of acquired tumor radioresistance and could be a potential target for pharmacological manipulations in the anti-cancer therapy.

641 POSTER

Inhibition of PDGF signaling attenuates radiation-induced pulmonary fibrosis

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Background: Pulmonary fibrosis is the consequence of a variety of diseases with often poor prognosis and no satisfactory treatment option. Fibrosis is also a common, delayed side effect of radiation therapy. Given new insights into cytokine signaling in the pathogenesis of fibrosis we sought to investigate the role of PDGF signaling in a radiation-induced lung fibrosis model.
Methods: The thoraces of C57BL/6 mice were irradiated, and the PDGF receptor (PDGFR) kinase inhibitor, SU9518, or vehicle were administered subcutaneously twice per week for 26 weeks. The progression of pulmonary fibrosis was monitored by high resolution CT and by histological examination. PDGFR phosphorylation status was demonstrated by IHC or IP/western blotting. A 2 chamber co-culture system was used to demonstrate radiation-induced endothelial cell PDGF expression.
Results: Administration of SU9518 potently inhibited the constitutive phosphorylation of PDGFR that was induced by total thoracic irradiation. Blockade of PDGF signaling markedly attenuated the development of pulmonary fibrosis and significantly increased survival of irradiated mice. We also demonstrated that radiation of endothelial cells stimulated sufficient PDGF expression to promote fibroblast proliferation, which was abrogated by SU9518.
Conclusions: Our data indicate that inhibition of fibrogenesis, rather than the anti-inflammatory response, is the key antifibrotic mechanism of the PDGFR kinase inhibitor. Our findings emphasize the pivotal role of PDGF signaling in the pathogenesis of pulmonary fibrosis. To our knowledge, this is the first report of an agent that can prolong survival in a pulmonary fibrosis model. The availability of new drugs which affect PDGF signaling makes these findings significant to current therapeutic approaches to pulmonary fibrosis and potentially to fibrosis in other organs and of various pathogenesis.

642 POSTER

Molecular targeting of epidermal growth factor receptor (EGFR) positive gliomas for neutron capture therapy using boronated bioconjugates

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Boron neutron capture therapy (BNCT) is based upon the nuclear capture and fission reactions that occur when non-radioactive ^{10}B is irradiated with low energy neutrons to produce high energy α particles (^{10}B[n,α]^{7}Li). In order for BNCT to be successful, a sufficient amount of ^{10}B (~20 μg/g tumor) and neutrons must be delivered to the tumor. The purpose of