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.

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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.

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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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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