Material and methods: Cytotoxic activity (IC50 and IC90) was tested by colorimetric SRB assay. In subsequent assays cell lines were treated with equitoxic IC90 doses of THPC11PtCl2 and CDDP. Drug uptake studies were performed using atomic absorption spectroscopy. Determination of apoptotic cell death was proven by DNA fragmentation and cleavage of PARP. Regarding the regulation of apoptosis induction, activation of caspase-3 was examined by western blotting and substrate cleavage kinetics. Furthermore treated and untreated cells were investigated for cytochrome c release, p53 expression and cell cycle analysis. Representing the induction of structural alterations of DNA cells were subjected to DNA-gelelectrophoresis.
Results: The cell line 1411HP showed a 3.3-fold CDDP-resistance as compared to H12.1 by respective IC90 values, which could completely overcome by treatment of cells with THPC11PtCl2. Measurements of platinum uptake revealed a higher accumulation of THPC11PtCl2 as compared to CDDP in both cell lines. Moreover THPC11PtCl2 was 2-fold more enriched in resistant 1411HP than in H12.1. Treatment with both agents resulted in a similar release of cytochrom c and cleavage of caspase-3 and PARP. However, after exposure to THPC11PtCl2 activation of caspase-3 was accelerated and no upregulation of p53 was observed. In addition, pre-treatment with the caspase inhibitor Z-VAD-Fmk did not inhibit apoptosis induction. THPC11PtCl2 treatment led to a DNA-mobility different from CDDP and induced no cell cycle arrest.
Conclusions: Our results revealed a selective higher activity and an increased drug accumulation of THPC11PtCl2 in the CDDP-resistant TGCT-cell line 1411HP. Considering the different efficacy and mechanism of apoptosis induction bypassing the caspase- and cell cycle-dependent apoptotic pathway, THPC11PtCl2 could serve as a new promising anti-cancer agent.

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A phase II study of daily gefitinib plus weekly paclitaxel (GP) in Taiwanese non-small cell lung cancer (NSCLC) patients who failed prior gefitinib or both gefitinib and taxane treatment

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Background: Gefitinib (G) is an active agent in 27% of Taiwanese NSCLC patients. GP has demonstrated anticancer activity in patients who failed G.
Purpose: To evaluate the activity and toxicity of GP in NSCLC patients who had previously failed treatment with G or with both G and taxane (G/T).
Methods: Eligibilities were histologically and/or cytologically proven NSCLC failed G (refractory or resistant), measurable lesion, ECOG PS0–3, adequate organ function, life expectancy longer than 6 weeks and written informed consent. They were chemonaive or had failed prior chemotherapeutic regimen(s). Oral G (250 mg) daily and intravenous P (60 mg/m^2) d1, 8, 15 were administered and repeated every four weeks. Primary endpoint was response rate (RR) and secondary endpoints were disease control rate (DCR), time to progression (TTP), overall survival (OS) and toxicity.
Results: From Sep 2004 to Sep 2005, 33 pts were enrolled and deemed eligible: M/F 16/17; median age 64; PS 1/2/3 13/17/3; stage IIIB/IV 3/30; adeno/squamous/NS 25/3/5. All 33 patients failed G, of them 4 were chemonaive, 8 had prior G and non T agents, 21 had prior G/T (docetaxel 13, paclitaxel 11), and 24 had prior platinum. Median prior chemotherapy regimen was 2 (0–7). A total of 281 cycles (median 4, range 1–18) were given. RRs were 24% and 29%, DCRs 48% and 43%, TTP 96 days and 45 days, median OS 264 days and 182 days for the overall and G/T groups, respectively. Grade 3/4 toxicities were leukopenia 6% and 5%, anemia 3% and 5%, Grade 3 flu-like symptoms 30% and 30% in overall and G/T groups, respectively. There was one treatment-related death.
Conclusion: This GT regimen showed a moderate activity and low toxicity as salvage treatment in patients who previously failed G or G/T treatment.

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Combined modalities of resistance in an oxaliplaitin-resistant human gastric cancer cell line with enhanced sensitivity

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Background: In order to identify mechanisms underlying oxaliplatin resistance, a subline of the human gastric adenocarcinoma TSGH cell line was made resistant to oxaliplatin by continuous selection against increasing drug concentrations. In present study, we would like to investigate the biochemical and molecular mechanisms through which cells acquire oxaliplatin resistance.
Materials and Methods: The *in vitro* IC_{50} and LC_{50} values were examined by the methylene blue dye assay and clonogenic survival assay, respectively. GSH/GSSG assay, platinum accumulation assay, platinum-DNA adduct assay, host cell reactivation assay, RNA interference technique, RT-PCR, and Western blotting were used to reveal molecular events in this study.
Results: Compared with the parental TSGH cells, the S3 subline showed 58-fold resistance to oxaliplatin; it also displayed 11-fold and 2-fold resistance to cis-diammine-dichloroplatinum (II) (cisplatin, CDDP) and copper sulfate, respectively. Interestingly, S3 cells were 4-fold more susceptible to 5-fluorouracil-induced cytotoxicity. Western blot analysis showed increased copper transporter ATP7A level and decreased thymidylate synthase level in S3 cells compared with TSGH cells, but the levels of ATP7B were identical. Cellular CDDP accumulation was significantly decreased in S3 cells, whereas oxaliplatin accumulation was similar for both lines. Amounts of oxaliplatin-DNA and CDDP-DNA adducts in S3 cells were about 15% and 40% of levels seen with TSGH cells, respectively. Resistance indexes between S3 and TSGH cells to oxaliplatin and CDDP were both reduced by approximately half when cells were pre-treated with P-type ATPase-inhibitor sodium orthovanadate. Despite elevated glutathione levels in S3 cells, there was no alteration of resistant phenotype to oxaliplatin or CDDP as measured by clonogenic assay when cells were co-treated with L-buthionine-(S,R)-sulfoximine. Host reactivation assay revealed enhanced repair of oxaliplatin-damaged and CDDP-damaged DNA in S3 cells compared with TSGH cells.
Conclusions: Together, our results show that the mechanism responsible for oxaliplatin and CDDP resistance in S3 cells is the combination of increased DNA repair and overexpression of ATP7A. Downregulation of thymidylate synthase in S3 cells renders them more susceptible to 5-fluorouracil-induced cytotoxicity.

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Acquired resistance to oxaliplatin in colon cancer cell lines is associated with up-regulation of G2/M checkpoint

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Oxaliplatin has a major role in the treatment of colorectal cancer, and a greater understanding of determinants of sensitivity and resistance is needed. To investigate the molecular basis of acquired resistance, we isolated oxaliplatin-resistant HCT116 and HCT116p53$^{-/-}$ colon cancer sub-lines (HCT.ORC21 and HCTp.ORC17, respectively) by repeatedly exposing parental cells to gradually increasing concentrations of the drug. The IC_{50} concentrations of oxaliplatin established in MTT assays are 25 mkM for HCT.ORC21 and 35 mkM for HCTp.ORC17, demonstrating a significant increase above those of parental HCT116 and HCT116p53$^{-/-}$ cells: 35- and 7-folds, respectively. A significant decrease in the cytotoxicity of oxaliplatin to the resistant cell lines was also observed in colony-forming assays. The resistant cell lines did not exhibit cross-resistance to TRAIL, and demonstrated on average only a 2-fold increase in resistance to cisplatin and 5-fluorouracil, suggesting that impairment of classical apoptotic pathways likely was not the major cause.
Assessment of p53 function in HCT.ORC21 cells suggested the selection for cells with impaired p53 function as one of the mechanisms of acquired oxaliplatin resistance: elevated levels of p53 protein and of its phosphorylation in response to oxaliplatin, loss of p21 and mdm2 induction, and the deregulation of G1 arrest in HCT.ORC21 cells, were all consistent with p53 mutation.
The much lower proliferation rate of both oxaliplatin-resistant cell lines prompted us to evaluate activation of checkpoints and cell cycle responses to oxaliplatin in the whole panel. In HCT116 cell line both expression and activation of Chk1 were abrogated by 48 hours of oxaliplatin treatment, whereas cell lines with impaired p53 demonstrated persistent expression and activation of Chk1 in response to prolonged drug exposure. HCTp.ORC17 cells demonstrated higher sensitivity to Chk1 inhibitor SB218078, both as a single agent and in combination with oxaliplatin, in colony-forming assays than HCT.ORC21 cells, suggesting a critical role of Chk1 in p53 negative genetic background. Also, oxaliplatin-resistant cell lines showed higher continuous expression of cyclin B, as compared to parental cell lines, pointing to profound G2/M arrest upon drug treatment. Our data point to persistent activation of Chk1 and G2/M block in response to drug as characteristics of cells with acquired resistance to oxaliplatin.