chemotherapy. With dose-limiting myelosuppression occurred in only HP pts at 700 and 800 and mainly gr 1–2 toxicity at 610, the recommended phase II dose (RD) for HP is proposed at 610. To further define toxicity profile and RD for LP pts, accrual is ongoing at 800. PK at 800 will be available at the meeting. Weekly 1-h infusion of Trabectedin seems to be convenient, active and well tolerated.

551 POSTER

Irofulven (IROF) enhances the antiproliferative effects of oxaliplatin (oxa) in human colon and breast cancer cells

M. Serova[1], F. Calvo[1], E. Cvitkovic[2], F. Koeppel[3], V. Poindessous[3], A.K. Larsen[3], E.S. Van Laar[4], S.J. Waters[4], E. Raymond[2]. [1]*Hopital Saint-Louis, Laboratoire de Pharmacologie Experimentale et Clin, Paris, France;* [2]*Hospital Saint-Louis, Department of Oncology, Paris, France;* [3]*Ecole Normale Supérieure and Institut Gustave-Roussy, Group of Biology and Pharmacogenetics of Human Tumors, Cachan and Villejuif, France;* [4]*MGI Pharma Inc, Bloomington, MN, USA*

Background: IROF (6-hydroxymethylacylfulvene, MGI-114) is a novel DNA-interacting anticancer drug derived from the mushroom natural product illudin S. IROF displays activity against human tumors *in vitro* and *in vivo*, and clinical trials as a single agent and in combination with several other anticancer drugs are underway. This study examined the cytotoxicity by combining IROF with OXA, a DACH-platinum compound demonstrating clinical activity in a wide variety of tumors including colorectal cancers. **Materials and Methods:** Drug interaction studies were performed using the Chou & Talalay method in a panel of human colon and breast cancer cell lines. **Results:** Single agent IROF displayed cytotoxicity against human colon cancer HT29 cells (IC50: 1.3±0.2 μg/mL), HT29 IROF-resistant IF2 cells (IC50: 92±9 μg/mL) and human breast cancer MCF7 cells (IC50: 2.0±0.2 μg/mL), HT29 being the most sensitive. In HT29, the OXA-IROF combination led to clear evidence of synergistic activity (Figure 1).

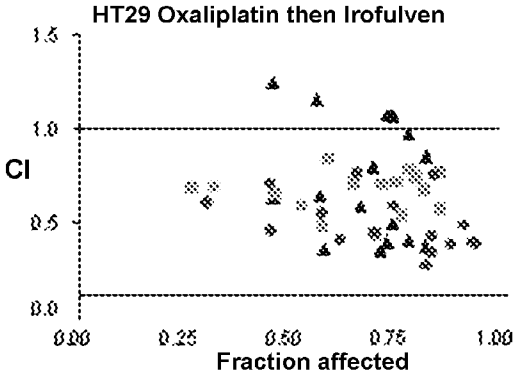

Figure 1. Combination indexes (CI) of IROF-OXA combinations in HT29 colon cancer cells: each spot represents one experiment performed in triplicate (shapes distinguish three separate experiments).

OXA given prior to or after IROF, consistently led to CI<1, demonstrating synergy. Similarly, simultaneous exposure to IROF and OXA was associated with synergy. Similar experiments were done in HT29 IROF-resistant IF2 cells. Acquired resistance to IROF slightly decreased sensitivity in this cell line to the IROF-OXA combination. Additive anti-proliferative effects were observed at low concentration IROF-OXA combinations; whereas, synergy was seen at higher concentrations. From our results, the sequence OXA followed by IROF appears to be the most efficient. To validate the experiments in colon cancer HT29 cells, we explored these combinations in MCF-7 breast cancer cells. In this cell line, additive and/or synergistic effects were observed when OXA was given after or concomitantly to IROF. Only additive effects were observed when OXA was given prior to IROF. **Conclusion:** IROF displays synergistic anti-proliferative effects when combined with OXA over a broad range of concentrations in human colon and breast cancer cells. Acquired resistance to IROF has limited impact on the effects of the combination. Based on these data, the IROF-OXA combination will be further explored in clinical trials, preferably using an OXA prior to IROF schedule.

552 POSTER

Genetic alterations and histology are related to the distinct responses of xenografted gliomas to different alkylating agents

P. Leuraud[1], L. Taillandier[2], J. Medioni[3], J.-Y. Delattre[4], M. Sanson[4], M.-F. Poupon[1]. [1]*Institut Curie, FRE 2584, Paris, France;* [2]*Faculté de Médecine, Neurologie, Nancy, France;* [3]*Hôpital St Louis, Centre d'Investigation Clinique, Paris, France;* [4]*Groupe Hospitalier Pitié-Salpêtrière, Féderation de Neurologie Mazarin, Paris, France*

Background: Response of gliomas to chemotherapy varies widely according to their histological subtype and grade. GBM being resistant while oligodendrogliomas are more chemosensitive. Recent studies showed that, in oligodendrogliomas, the double loss of chromosome 1p and 19q was related to an overall better prognosis. In contrast, several studies have identified genetic alterations related with a poor prognosis, such as EGFR amplification, PTEN mutation or 10q loss, CDKN2A homozygous deletions. Nevertheless, no clear correlation with chemoresistance has been established.
To further study the molecular alterations underlying response to chemotherapy, a series of 12 human gliomas, derived from surgical specimens, was established as xenografts in *nude* mice and used to evaluate *in vivo* the relationship between histology, genetic parameters and response to alkylating drugs generally used in malignant gliomas, BCNU, Carboplatin (CP), Ifosfamide (IFO), and Temozolomide (TMZ).
Material and methods: Of the 12 xenografts used, 8 were high-grade oligodendroglial tumors and 4 were GBM. They were characterized for their genetic alterations, including those considered as "early" alterations, namely chromosome 1 loss +/− chromosome 19q loss, TP53 mutation, and those considered as "late" alterations, namely chromosome 10 loss, chromosome 9p loss, EGFR genomic amplification, PTEN mutation, CDKN2A homozygous deletion and telomerase reactivation. Chemosensitivity to 4 alkylating agents, TMZ {42 mg/kg, d1–5 per os (p.o.)}, BCNU {(5 mg/kg, d1 intraperitoneal (i.p.)}, IFO {90 mg/kg, d1–3, i.p.}, and CP {66 mg/kg, d1, i.p.} was tested.
Results: Although each tumor presented an individual response pattern, GBM had a lower chemosensitivity than oligodendrogliomas and TMZ was the most effective drug. Deletion of 1p+/−19q was associated with higher chemosensitivity, while late molecular alterations, particularly EGFR amplification were associated with chemoresistance.
Conclusions: These results suggest that the combined use of histology and molecular markers should eventually be helpful to select the most appropriate agents in malignant oligodendrogliomas and astrocytomas.

553 POSTER

Activation of trans geometry in bifunctional mononuclear platinum complexes by combining aliphatic and aromatic amines. Mechanistic studies on antitumor action

C. Navarro-Ranninger[1], F.J. Ramos-Lima[1,2], O. Vrana[2], O. Novakova[2], A.G. Quiroga[1], V. Brabec[2]. [1]*Universidad Autónoma, Química Inorgánica, Madrid, Spain;* [2]*Academy of Sciences, Institute of Biophysics, Brno, Czech Republic*

The global modification of mammalian and plasmid DNAs by novel platinum compounds, *trans*-[$PtCl_2$(Am1)(Am2)], where Am1 = isopropylamine and Am2 = 3-hydroxymethyl or 4-hydroxymethyl was investigated in cell-free media using various biochemical and biophysical methods. These modifications were analyzed in the context of the activity of these new compounds in several tumor cell lines including those resistant to antitumor *cis*-diamminedichloroplatinum(II) (cisplatin). The results showed that the replacement of both ammine group in clinically ineffective trans isomer of cisplatin [*trans*-diamminedichloroplatinum(II) (transplatin)] resulted in a radical enhancement of its activity in tumor cell lines so that these analogues were more cytotoxic than cisplatin and exhibited significant antitumor activity including activity in cisplatin-resistant tumor cells. Importantly, this replacement also markedly altered DNA binding mode of transplatin. The results offer a strong experimental support for the view that one strategy how to activate trans geometry in bifunctional platinum(II) compounds and to circumvent resistance to cisplatin consists in a chemical modification of the conventional transplatin which would result in their increased efficiency to form in DNA interstrand cross-links. The present work also suggests that such a modification may be accomplished by the replacement of both ammine groups by aliphatic amine ligands, such as isopropylamine and 3-hydroxymethyl or 4-hydroxymethyl. Moreover, the analogues of transplatin apparently represent a novel class of platinum anticancer drugs acting by a different mechanism than "classical" cisplatin.
Acknowledgments
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Johnson Matthey PLC is also acknowledged for their generous gift of K_2PtCl_4.

554 POSTER

A broad panel of screening assays for mutation and methylation assessment of genes involved in the pathological development and therapeutic treatment of NSCLCa broad panel of screening assays for mutation and methylation assessment of genes involved in the pathological development and therapeutic treatment of NSCLC: utility for therapeutic monitoring

S. Lilleberg, J. Durocher, C. Zhi, C. Sanders, B. Walters, M. Geimer, J. Hempel, D. Foster, P. Fogle, C. Wasserburger. *Transgenomic, Translational and Clinical Research, Omaha, USA*

Non-small cell lung cancers (NSCLC) contain numerous alterations, including somatic mutations and CpG island hypermethylation in a variety of genes that contribute to the pathological phenotype. Some of these tumor-associated variations have also been detected in the DNA found in the plasma of NSCLC patients, including DNA with mutations in TP53 and KRAS, and hypermethylation in p16INK4a and MGMT. However, a more comprehensive study designed to assess both mutation and methylation status of NCSLC patients at various stages has not been reported. Here we describe a combined assay panel for the assessment of genes associated with disease pathogenesis and therapeutic treatment of NSCLC. DNA from primary tumors and matching patient plasma were screened for mutations in a variety of genes including PTEN, TP53, TP73, KRAS, NRAS, BRAF, MET, EGFR, PDGFRA, PDGFRB, and KIT, and alterations of methylation status in APC, MLH1, p16, p14, MGMT, GSTP1, DAPK, RASSF1A, RUNX3, RARB2, and FHIT. We chose mutation and methylation scanning technology and these targets to develop a thorough screening methodology for alterations known to be associated with NSCLC or that may be involved in resistance to targeted therapeutics. Variations were identified with a denaturing high-performance liquid chromatography (DHPLC) platform that uses post-separation fluorescence technology, enabling the detection of variants that represent <0.1–1.0% of the total analyzed DNA. Using this approach, we identified at least one somatic or epigenetic event in 100% of the NSCLC patients. In no case was a mutation found in the primary tumor that was not also present in the plasma. The results emphasis the heterogenous pattern of genomic alterations and that scanning provides an attractive approach to comprehensive NSCLC genetic and epigenetic screening. The thoroughness of approach may have important implications for screening and staging, and disease monitoring during and following therapy.

555 POSTER

Metabolic activation of satraplatin by haemoglobin in vitro

M. McKeage, J. Carr, M. Tingle. *The University of Auckland, Dept of Pharmacology and Clinical Pharmacology, Auckland, New Zealand*

Background: Satraplatin is an orally administered Pt(IV) drug that has shown clinical activity against advanced prostate and other cancers. Satraplatin and other platinum-based drugs are known for undergoing chemical transformation in the blood steam but the role of haemoglobin in this process is unclear. In the present study, we characterised the reactions occurring between satraplatin and haemoglobin *in vitro*. Methods: The satraplatin concentration of reaction solutions was determined by a satraplatin-specific HPLC-ICPMS assay. The stability of satraplatin was determined in haemoglobin solutions under various conditions by changing the ligandation and oxidation state of haemoglobin, availability of co-factor and other experimental variables. Results: In solutions containing met-haemoglobin, satraplatin was completely stable. However, in solutions containing reduced haemoglobin and NADH, satraplatin was rapidly converted ($t_{1/2}$, 35 mins) to its major Pt(II) active metabolite (JM118). Visible spectrometry of the reaction solution revealed increasing absorption at 500 and 630 nm and decreasing absorption at 542 and 576 nm, characteristic of the conversion of oxy-haemoglobin to met-haemoglobin. Factors affecting the reaction between satraplatin and haemoglobin were investigated. Cold temperature, carbon monoxide, oxygen and the absence of co-factor inhibited the haemoglobin-catalysed metabolism of satraplatin. Conclusion: Haemoglobin catalysed the activation of satraplatin by a mechanism involving electron transfer from haem-iron to drug molecules, resulting in the reduction of the parent Pt(IV) drug to active Pt(II) metabolites concurrently with the oxidation of haeme iron from Fe(II) to Fe(III). Inhibitor studies suggested that reduced unliganded haem-iron is required for the haemoglobin-catalysed metabolic activation of satraplatin.
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556 POSTER

Histone deacetylase inhibitors induce chromatin decondensation by down-regulation of proteins involved in the maintenance of heterochromatin

D.C. Marchion, E. Bicaku, A.I. Daud, D.M. Sullivan, P.N. Munster. *H. Lee Moffitt Cancer Center, Interdisciplinary Oncology, Tampa, USA*

Background: Genome stability and transcriptional silencing are accomplished by condensation of the chromatin in the form of heterochromatin. The maintenance of heterochromatin is regulated by hypermethylation and hypoacetylation of DNA and histones. Histone acetylation attenuates the electrostatic interactions between histones and DNA. This may loosen the chromatin structure. This study evaluates the effects of histone deacetylase inhibitors (HDACi) on chromatin structure and dynamics and investigates a mechanism by which HDACi may increase the susceptibility of cancer cells to DNA damaging agents.
Methods: Changes in the chromatin structure were evaluated by electron microscopy, DNA hypersensitivity assays, and DNA binding assays. The effects of HDACi on protein expression were evaluated using microarray, Northern and Western blot experiments.
Results: We found that exposure of breast cancer cells to the HDACi, valproic acid, suberoylanilide hydroxamic acid, sodium butyrate and trichostatin A resulted in histone hyperacetylation and chromatin decondensation. While maximal histone acetylation occurred within one hour, chromatin decondensation required prolonged exposure to an HDACi for at least 48 hours, suggesting that histone hyperacetylation alone may not be sufficient for chromatin decondensation. We therefore evaluated the effects of HDACi on other factors involved in the epigenetic control of chromatin dynamics. A down-regulation in the expression of several family members of the structural maintenance of chromatin (SMC) proteins and SMC-associated proteins, DNA methyltransferase and heterochromatin proteins was observed after 48 hours exposure to an HDACi. This correlated with enhanced sensitivity of DNA to nucleases and increased interaction of DNA with intercalating agents. The HDACi-induced chromatin decondensation correlated with enhanced DNA damage and apoptosis induced by DNA damaging agents such as topoisomerase I and II inhibitors.
Conclusions: HDACi-induced histone hyperacetylation alone is not sufficient for chromatin decondensation, but is associated with the down-regulation of gene products involved in the maintenance and dynamics of heterochromatin. The HDACi-induced chromatin decondensation may facilitate access of DNA damaging agents to their target sites and thereby enhances their efficacy.

557 POSTER

Evidences for G-quadruplex formation in the mitochondrial genome

N. Dias[1], J.F. Goossens[2], L. Lacroix[3], J.L. Mergny[3], F. Rosu[4], P. Colson[4], M.P. Teulade-Fichou[5], A. Lansiaux[1], C. Bailly[1]. [1]*INSERM U-524, Lille, France;* [2]*Faculté des Sciences Pharmaceutiques et Biologiques, Lille, France;* [3]*Museum National d'Histoire Naturelle, Paris, France;* [4]*University of Liège, Liège, Belgium;* [5]*Collège de France, Paris, France*

G-quadruplex (G4) DNA sequences, as found in human telomeres and certain oncogenes (e.g. c-*myc*), are considered as potential targets for anticancer agents. Various G4 sequences have been identified in the nuclear genome. Here we show that such folded sequences can also be found in the mitochondrial genome. Mitochondrial DNA (mtDNA) is a compact circular double-stranded DNA of 16.6 kb with few intergenic sequences and no intron, and presents G rich (Heavy) and C rich (Light) strands. mtDNA mutations have been characterised in various types of human cancers and these mutations may contribute to carcinogenesis or tumor cell proliferation. It is therefore important to identify regulatory sequences in the mtDNA. In our study, G-rich oligonucleotides corresponding to defined regions in the mitochondrial genome were evaluated for their ability to form G-quadruplex structures. Using different spectroscopic approaches, we demonstrate that two oligonucleotides, Mt2 and Mt4, were able to form G-quadruplexes which stability depends on the nature of the mono-cation. We also investigated the binding of the bis(quinacridine) macrocyclic compound BOQ1 to these sequences by electrospray mass spectrometry and fluorescence studies. We show that this compound, previously described as a G-quadruplex telomeric DNA binding molecule, interacts selectively with the two quadruplexes Mt2 and Mt4. The identification of different regions in the mitochondrial genome potentially capable to form G-quadruplexes provides opportunities to target these sequences with small molecules.