part of the *in vivo* therapeutic response could be due to a direct anti-angiogenic effect of irinotecan. This hypothesis is in agreement with the predominant cytostatic effect we observed *in vivo*.

446 POSTER

ST1968: a new camptothecin analogue endowed with distinctive pharmacological properties

C. Pisano, L. Vesci, F. Bucci, M. Guglielmi, A. Marconi, F.F. Ferrara, R. Foderà, C. Aulicino, S. Zavatto, P. Carminati. *Sigma-Tau S.p.A., Oncology-R&D, Pomezia (Rm), Italy*

Camptothecins isolated from the Chinese tree *Camptotheca acuminata* are effective antitumor drugs acting as topoisomerase I poisons. Although camptothecin derivatives topotecan and irinotecan are currently used in clinic, their narrow therapeutic index as well as the percentage of "naïve resistant patients" have generated interest in developing new camptothecin analogues with an improved pharmacological profile. A preliminary screening of various new camptothecin derivatives in a yeast model of Saccharomyces cerevisiae expressing wild type or different camptothecin-resistant mutants of human DNA topo I, allowed us to characterize a molecule, ST1968, able to inhibit the viability of yeasts transformed with wild type and mutated DNA topo I. ST1968 revealed *in vitro* an antiproliferative activity at nanomolar doses for lung (NCI-H460), ovarian (A2780) and prostate (DU145) carcinomas, and at micromolar doses for squamous oral carcinoma (KB) and other cancers. Flow cytometry showed that ST1968 exerted on tumor cells mainly a cytostatic activity by arresting cells in G_2/M upon a 2h exposure, and both cytostatic and cytotoxic activities with cell cycle block in G_2/M and induction of apoptosis upon 72h. In all *in vitro* experiments, the irinotecan metabolite SN38 appeared to be more cytotoxic than ST1968. Conversely, in *in vivo* studies, ST1968 delivered intravenously according to the schedule q4dx4, revealed a high efficacy against rapidly growing tumors (A2780, KB) as well as a camptothecin-resistant slowly growing human solid tumor (DU145-RC1). Interestingly, the efficacy of ST1968 in terms of tumor volume inhibition (TVI%) or log10 cell kill (LCK), complete response (CR) and Long Term Survivors (LTS) was substantially improved compared to irinotecan. *In vitro* uptake and release studies exhibited a direct correlation between cytotoxicity and intracellular concentrations of ST1968 in A2780 and DU145 cells, while in KB cells a very low cytotoxicity was associated to very high drug concentrations. *In vivo*, the highest AUCinf was found in A2780 and KB tumor lesions and a correlation between tumor uptake and antitumor activity of ST1968 was observed.
In conclusion, the high ST1968 antitumor activity toward a broad range of tumors, including camptothecin-resistant tumor expressing mutated topoisomerase I, and its higher therapeutic index compared to irinotecan support the clinical investigation of this new drug.

447 POSTER

The impact of UGT1A1*28 and UGT1A1*6 on irinotecan-induced neutropenia in Asian cancer patients receiving weekly and three weekly irinotecan regimens

B. Chowbay[1], S.J. Srinivasa Jada Rao[1], B.C. Goh Boon Cher[2], R. Robert Lim[2], W.T. Lim Wan Teck[3], E.H. Tan Eng Huat[3]. *[1]National Cancer Centre, Clinical Pharmacology Laboratory, Division of Medicine, Singapore, Singapore; [2]National University Hospital, Department of Haematology–Oncology, Singapore, Singapore; [3]National Cancer Centre, Department of Medical Oncology, Singapore, Singapore*

Background: Irinotecan is a topoisomerase I inhibitor and its pharmacokinetics and pharmacodynamics are greatly influenced by several polymorphic variants in genes responsible for encoding the various drug metabolizing enzymes and drug transporters involved in its disposition. Severe diarrhoea and neutropenia are two dose-limiting toxicities of irinotecan and its incidence varies in cancer patients of different ethnic background and depends on the *UGT1A1* polymorphic status. The purpose of this study was to investigate the influence of *UGT1A1*28* and *UGT1A1*6* polymorphisms on toxicity in Asian cancer patients receiving either the weekly or three weekly schedules of irinotecan.
Materials and Methods: Patients received irinotecan infusion either at 100 mg/m^2 on days 1, 8 and 15 and the regimen was repeated every 28 days (N = 28) or at 375 mg/m^2 once every three weeks (N = 46) over 90-minutes. A total of 19 and 18 serial blood samples were collected from cancer patients in the weekly and three weekly schedules, respectively, on the first day of irinotecan administration. The promoter (1.5 kb) and exon 1 of the *UGT1A1* gene was screened for polymorphic variants (N = 73) and correlated with irinotecan's pharmacodynamic parameters.
Results: A significantly higher exposure levels to irinotecan ($AUC_{0-\infty}$/dose/BSA: 42.1$\pm$18.8 vs 14.5$\pm$6.4; P = 0.0001) and SN-38 ($AUC_{0-\infty}$/dose/BSA: 1.2$\pm$0.63 vs 0.71$\pm$0.39; P = 0.0002) and lower exposure levels to SN-38G ($AUC_{0-\infty}$/dose/BSA: 4.2 $\pm$ 2.4 vs 10.1 $\pm$ 4.0; P = 0.0001) were observed in cancer patients receiving the three weekly regimen compared with patients on the weekly regimen. The relative extent of glucuronidation (REG) was approximately 5-fold lower in cancer patients on the three weekly regimen compared with those on the weekly regimen (REG: 3.9$\pm$2.2 vs 18.3$\pm$13.9; P = 0.0001). The *UGT1A1*28* and *UGT1A1*6* alleles were present in 20% and 13% of the cancer patients, respectively. Diarrhoea was uncommon in cancer patients receiving either schedules of irinotecan. Grade 4 neutropenia (ANC $\leqslant$ 500/μL) occurred in 26% (N = 12) of cancer patients on the three weekly schedule. None of these patients were homozygous for the *UGT1A1*28* allele. One patient was heterozygous and one was homozygous for the *UGT1A1*6* allele while the rest carried the reference genotype.
Conclusion: This study showed that neutropenia was more common in cancer patients receiving the three weekly irinotecan regimen compared with patients on the weekly regimen and this effect may be attributed to the high systemic exposure levels to SN-38 in patients receiving the three weekly regimen. The *UGT1A1*28* and *UGT1A1*6* variants did not have significant impact on the incidence of neutropenia in the Asian cancer patients and questions the need for *UGT1A1* testing in Asian cancer patients receiving irinotecan. Perhaps the less toxic but effective weekly schedule of irinotecan should be adopted in Asian cancer patients.

Topoisomerase II inhibitors

448 POSTER

Common over-expressions of TOP2A in hepatocellular carcinoma: a potential therapeutic target

N. Wong[1], N.L. Wong[1], B. Beheshti[3], J. Squire[3], K. Chan[1], P. Lai[4]. *[1]Chinese University of Hong Kong, Anatomical and Cellular Pathology, Hong Kong, China; [2]Chinese University of Hong Kong, Clinical Oncology, Hong Kong, China; [3]University of Toronro, Medical Physics, Toronto, Canada; [4]Chinese University of Hong Kong, Surgery, Hong Kong, China*

Genomic amplification is common in human cancers and represents an important mechanism in the activation of proto-oncogenes. In many instances, over-expressed oncogenes induced from genomic gains hold clinical implications both as prognostic markers and targets for therapeutic design. In this study, regional genomic gains that are commonly found in hepatocellular carcinoma (HCC) were investigated for underlying over-expressed genes by transcriptional mapping. Using a high-density cDNA microarray, a series of 22 HCC cell lines was screened for candidate genes at 8 loci. A subset of consistently over-expressed genes was indicated in each of the genomic locus, which included hepatoma derived growth factor (at 1q21.3), C-MYC (at 8q24.2) and aurora kinase A (at 20q13.2). Distinctively, common over-expressions of the DNA topoisomerase II alpha gene (TOP2A; at 17q21.2) ranked the highest with an average induction of 18.5-fold. By FISH analysis, a concordant copy gain of TOP2A was confirmed in most cell lines (~80%). Quantitative RT-PCR revealed the presence of frequent TOP2A up-regulations in HCC tumours compared to paired adjacent non-malignant liver tissues (p = 0.0018). Since Etoposide (a topoisomerase II binding agent) can interrupt the activity of DNA topoisomerase II by suppressing the enzyme mediated DNA cleavage, we investigated the potential therapeutic value in targeting TOP2A by Etoposide, as a single agent, and in combination with Doxorubicin (a DNA intercalator), which is currently the first-line chemotherapeutic agent for HCC patients. *In-vitro* cytotoxic studies on HCC cell lines suggested a potent synergistic effect in the combinatory application of Etoposide with Doxorubicin. Etoposide applied at IC20 concentrations readily reduced the IC50 concentrations of Doxorubicin by 3–5 folds when compared to Doxorubicin alone. Our study is the first to demonstrate a synergistic effect in the combinatory application of Etoposide with Doxorubicin and highlights the potential use of TOP2A reactive agents in the clinical treatment for patients with HCC.