from paraffin wax embedded tissues from these cases for the expression of oestrogen receptor (ER), preogesterone receptor (PR), human epidermal growth receptor 2(c-erbB-2 or HER2/neu), p53 and cyclin D1 (CCND1) was carried out using the avidin biotin complex (ABC) procedure. This procedure is briefly as follows:
Sections were mounted on adhesive coated glass slides and deparaffinised in xylene. They were rehydrated in graded alcohol and placed in 0.5% hydrogen peroxide to quench endogenous peroxidase. Antigen retrieval was achieved by microwave oven incubation in citrated buffer (0.01M: pH 6.0) for ER, PR, c-erbB-2 and p53 oncoproteins and in 1mM EDTA (pH 8.0) for Cyclin D1. Antigen localisation was achieved by incubating sections with primary antibodies (polyclonal rabbit antihuman erbB-2 and monoclonal mouse antihuman ER, PR, p53 and CyclinD1) at various dilutions. The indirect avidin-biotin complex (ABC) procedure using 3,3-diaminobenzidine tetrahydrochloric (DAB) as the substrate chromogene was applied for detection of bound antibody.
The Chi square test was used for statistical analysis.
Results: Invasive ductal carcinoma made up 92.7% of the Nigerian patients compared to 77% seen in the British patients. Significant difference in clinical stage but not tumour grades was also observed.
The expression of ER and Cyclin D1 was significantly higher in the British patients than the Nigerian patients (X*=6.9143 P=0.0086, X*=4.9281 P=0.0234 respectively). Other markers showed no statistical difference.
Conclusion: The differences in prognosis of breast cancer between Nigeria and Britain may be partly explained by differences in hormone receptors and cell cycle regulation in addition to the obvious differences in stage at presentation

Telomerase-targeting agents

434 POSTER

The interactions of acyclic nucleotide analogues with human telomerase

M. Hájek, N. Matulová, I. Votruba, A. Holy. *Institute of Organic Chemistry and Biochemistry, Nucleic Acid Chemistry, Prague, Czech Republic*

Background: Purine acyclic nucleoside phosphonates (ANP) type PME {9-[2-(phosphonomethoxy)ethyl] derivatives of purines} and PMP {9-[2-(phosphonomethoxy)p ropyl] derivatives of purines} were shown as very potent antivirals active against DNA viruses and retroviruses. Moreover, these antimetabolites exhibit cytostatic activity *in vitro* and *in vivo*. Antiviral and cytostatic effects of these nucleotide analogues are the result of DNA polymerase and/or reverse transcriptase inhibition by their diphosphates (dNTP analogues). These findings led us to the idea to investigate their interactions with human telomerase, the reverse transcriptase capable of replacing the telomeric portion of the chromosome lost during DNA replication.
Material and Methods: ANP diphosphates were evaluated in telomeric repeat amplification protocol (TRAP) for their ability to inhibit the extension of telomeres by human telomerase, using extracts from human leukemia HL-60 cells as a source of the enzyme. Inhibition of telomerase was measured in the presence of various concentrations of studied ANPpp as inhibitors, and the natural dNTPs (125 $\mu mol \cdot l^{-1}$ each). The extent of the enzyme inhibition was expressed as IC_{50} values.
Results: Our data show that the most effective compound studied was the guanine derivative PMEGpp ($^{Tel}IC_{50}$ 12.7±0.5 $\mu mol \cdot l^{-1}$). The inhibitory effects of other PME and PMP diphosphates on telomerase reverse transcriptase decreased in the order: (*R*)-PMPGpp > PMEDAPpp > (*S*)-PMPGpp > (*S*)-HPMPApp > DAPympp > 6-cypr-PMPDAPpp > (*R*)-PMPApp > PMEApp > (*R*)-PMPDAPpp > (*S*)-PMPApp ≈ 6-Me_2-PMEDAPpp.
Conclusions: These results are consistent with the observed antineoplastic activities of the paternal PMEG and PMEDAP compounds. Moreover, structure-activity relationship indicates enantio-selectivity some of these human telomerase inhibitors. (*R*)-Isomers of the PMP-derivatives possess stronger affinity towards the enzyme than (*S*)-isomers. In accordance with human telomeric sequence, the adenine derivatives are less effective inhibitors than the guanine derivatives. The data contribute to the rational design of telomerase inhibitors based on the structure of acyclic nucleotide analogues.

435 POSTER

Biophysical, biological, and in silico investigation of 3,6,9-trisubstituted acridines targeting human telomeric G-quadruplex DNA

C. Schultes, M.J.B. Moore, B. Guyen, S. Neidle. *University of London School of Pharmacy, CRUK Biomolecular Structure Group, London, UK*

The activation of the holoenzyme telomerase is one of the key events in the oncological transformation of human cells (Hahn et al., 1999) and inhibition of telomerase has thus become established as a promising potential strategy for anti-cancer drug development in recent years. The single-stranded, G-rich telomeric DNA substrate of telomerase can form higher-order G-quadruplex (GQ) structures (Wang and Patel, 1997; Parkinson *et al.*, 2002), the detailed characterisation of which has allowed the application of rational drug design approaches to the development of small molecules that will have specificity for the unique features of this human GQ. Work in our group and others has thus resulted in the development of compounds that inhibit normal telomerase function in the nanomolar range by the targeting of telomeric GQ-DNA (Read *et al.*, 2001; Mergny *et al.*, 2002; Harrison *et al.*, 2003).
We shall report on the biophysical characterisation and initial biological evaluation of a set of 3,6,9-trisubstituted acridine derivatives as potent new telomerase inhibitors. The development of a number of compound series has allowed the establishment of clear structure-activity relationships (SARs) describing quadruplex-drug interactions, and current work is focused on elucidating the biochemical pathways activated upon cellular exposure to compounds selected in this manner. Screening of compounds for their ability to bind and stabilise the human telomeric GQ structure was carried out using a series of fluorescence resonance energy transfer (FRET)-based DNA melting experiments. Significant differences in the abilities of compounds to increase the melting temperature of GQ-DNA can be compared to enzyme inhibition data obtained from an *in vitro* cell-based assay of telomerase activity (TRAP assay), and this can then be translated directly into SARs that have enabled us to gain insights into the extent to which our *in vitro* assays allow the prediction of enzyme inhibition. A direct correlation between the GQ-stabilising ability of a compound and its ability to inhibit telomerase *in vitro* has been established in this way, both allowing the rapid screening of novel compounds with a high-throughput fluorescence method and lending support to the proposed mechanism of telomerase inhibition *via* GQ-stabilisation.
On-going studies aiming to elucidate in more detail the ligand-DNA interactions and the cellular response to compound exposure will also be presented. Molecular modeling approaches, including molecular dynamics simulations methods, are being used to rationalise the results from the biophysical assays on a molecular level, while changes in the cellular levels of telomerase and specific other telomere-associated and DNA-damage-response proteins are being investigated at the DNA expression level following exposure to our lead compounds.
Hahn, W.C., Counter, C.M., Lundberg, A.S., Beijersbergn, R.L., Brooks, M.W., Weinberg, R.A. (1999) Nature 400, 464–468.
Harrison, R. J., Cuesta, J., Chessari, G., Read, M. A., Basra, S. K., Reszka, A. P., Morrell, J., Gowan, S. M., Incles, C. M., Tanious, F. A., Wilson, W. D., Kelland, L. R., and Neidle, S. (2003) J. Med. Chem. 46, 4463–4476
Mergny, J. L., Riou, J. F., Mailliet, P., Teulade-Fichou, M. P., and Gilson, E. (2002) Nucleic Acids Res. 30, 839–65.
Parkinson, G. N., Lee, M. P., Neidle, S. (2002) Nature 417, 876–80.
Wang, Y., Patel, D. J. (1993) Structure 1, 263–82.

436 POSTER

The novel compound KML001 induces telomere attrition, senescence and chromosomal instability in cell lines with short telomeres

H.R. Hendriks[1], F. Dai[2], B. Rademaker[3], Y.J. Yang[4], S.B. Lee[4], A.M. Burger[5]. *[1]Hendriks Pharmaceutical Consulting, Purmerend, The Netherlands; [2]University of Freiburg, Department of Anatomy, Freiburg, Germany; [3]Rephartox B.V., Onstwedde, The Netherlands; [4]Korea Microbiological Laboratories, Kyonggi-do, Korea; [5]Sunnybrook & Women's College Health Sciences Centre, Molecular and Cellular Biology, Toronto, Canada*

Arsenic trioxide is experiencing a revival in cancer medicine since it has proven effective in the treatment of acute promyelocytic leukemia. Its mechanisms of action are currently being revisited to enable rational use of inorganic arsenic. Induction of apoptosis and reactive oxygen species, as well as striking effects on telomeres and telomerase have been described. The novel arsenic compound KML001 has shown preclinical activity in solid tumors and has just entered clinical trials. This study was initiated in order to investigate whether KML001 can target telomeres and telomerase. MCF-7 (6 kb), a human breast cancer cell line with longer telomeres, PC3 (3,5 kb) and UXF 1138L (2.5 kb), prostate and uterus cancer cell lines respectively with shorter telomeres, were chosen for *in vitro* experiments. The TRAP assay (telomeric repeat amplification protocol) was used to measure telomerase activity, ß-galactosidase staining for detection of cellular senescence, the sulforhodamine B assay for proliferation tests, Southern blotting to determine mean telomere fragment length (TRF), and fluorescence *in situ* hybridization (FISH) with human centromere and telomere probes to study chromosomal integrity. The IC_{50} for KML001 in PC3 cells was 1 μM, in MCF-7=4 μM, and in UXF 1138L=5 μM. KML001 treatment at doses around the IC_{50} potently shortened telomeres in PC3 and UXF 1138L, but not MCF-7 cells under continuous exposure.