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**Phase I and pharmacokinetic study of UCN-01 (U) in combination with irinotecan (I) in patients with solid tumors**

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**Background:** UCN-01,7-hydroxystaurosporine, inhibits serine-threonine kinases ($Ca^{2+}$ and phospholipid-dependent protein kinase C, cdks2,4,6, chk-1 and PDK1). UCN-01 mediates distinct effects *in vitro/in vivo*: cell cycle arrest in G1, abrogation of G2 arrest by inhibiting chk1, induction of apoptosis, and potentiation of cytotoxicity of S-phase active agents, like irinotecan (I). Due to this synergy and non-overlapping toxicity, U and I were combined in a phase I study, to determine the maximum tolerated dose (MTD), acute and chronic toxicity profile, and pharmacokinetics (PK) of U and I.
**Methods:** Patients with a histologically confirmed, incurable solid tumor refractory to standard therapy received I over 90 min on d1 and 8 of a 21d cycle. U was infused IV over 3 h on d1 immediately following I. All subsequent U doses were half the original dose. Starting doses of U and I were 50 and 60 mg/m$^2$, respectively. Initially, the U dose was increased in 20 mg/m$^2$ increments to its known MTD (90 mg/m$^2$); then the I dose would increase to 90 and 120 mg/m$^2$. Blood samples were collected cycle 1 for U, I, and three I metabolites (SN38, SN38G, and APC) for PK analysis. U, I, SN38, SN38G, and APC were quantitated by LC/UV and fluorescence. PK parameters were calculated by noncompartmental methods.
**Results:** A total of 9 patients with a variety of tumors have been enrolled on the trial at U/I doses of 50/60 mg/m$^2$ (n=1), 70/60 mg/m$^2$ (n=4) and 90/60 mg/m$^2$ (n=4). The long half-life ($t_{1/2}$) (541.1$\pm$320.2 h), low clearance (0.042$\pm$0.032 L/h), and volume of distribution (22.6$\pm$11.1 L) observed are consistent with prior UCN-01 data. While U PK does not appear to be affected by I, the reverse is not the case. There was a significant (P<0.01) decrease in $C_{max}$, AUC, and metabolite: I AUC ratio of I, SN38, SN38G, and APC, a significant (P<0.05) decrease in $t_{1/2}$ of I and APC, and an increase in SN38G exposure on d8 compared to d1. Stable disease has been documented in 4 patients, and 5 have been removed from the study (3 for PD, 1 for toxicity, and 1 by patient choice). No significant hematologic toxicity has been noted. The toxicities were hyperglycemia (2 G2, 1 G3), hyponatremia (1 G2), and hypocalcemia (2 G2). Two patients at the U/I 90/60 mg/m$^2$ dose level experienced a DLT (2 G3 hypophosphatemia).
**Conclusion:** The U/I 70/60 mg/m$^2$ dose level is being expanded, and escalation of I to single agent doses is planned. I dose escalation is warranted by the decrease exposure to I and SN38.

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**Phase I and pharmacokinetic study of indisulam (E7070) in combination with carboplatin, a CESAR Central European Society for Anticancer Drug Research – EWIV report**

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**Background:** Indisulam (E7070) is a novel sulphonamide derivative that induces dose- and time dependent G1 cell-cycle arrest as well as delay in G1/S transition and S phase progression, leading to G2 arrest followed by apoptosis. A unique anti-tumor spectrum and effects on cell cycle regulatory molecules such as cyclin E, cdk 2 and cyclin H indicate that E7070 has a novel mechanism of action when compared with existing therapies by the NCI COMPARE programme. A significant reduction in glutathione synthetase transcripts by indisulam provides a molecular basis for its combination with platinum agents. Indisulam demonstrated high activity against various non-small cell lung cancer models (NSCLC). When indisulam was tested as single agent therapy, every 3 weeks intravenously (i.v.), clinical results quite comparable to the established substances against NSCLC were observed.
**Objectives of this study were:** (1) to determine the recommended dose of indisulam in combination with carboplatin by dose adjustment, (2) to determine the pharmacokinetic profile of indisulam and carboplatin when administered in combination.
**Materials and Methods:** Patients with solid tumors refractory to standard therapy or for whom no established therapy exists, with a Karnofsky index *70%, a maximum of two previous lines of chemotherapy and normal essential organ functions were eligible. Based on prior pharmacokinetic investigations the dose of indisulam was based on the body surface area whereas that of carboplatin was calculated from the patient's glomerular filtration rate (GFR), both immediately prior to each cycle of therapy. GFR was obtained from the Cockroft-Gault formula. Indisulam was given as a one-hour i.v. infusion on day 1, carboplatin as an i.v. infusion over 30 minutes, after the end of indisulam on day 2 of a three-week cycle.
**Results:** The following dose levels (DL) were tested:
DL1: Indisulam 350 mg/m*, carboplatin AUC 6 mg/ml/min (3 pts; 6,7,6 cycles)
DL2: Indisulam 500 mg/m*, carboplatin AUC 6 mg/ml/min (3 pts; 5,2,1 cycles)
DL3: Indisulam 600 mg/m*, carboplatin AUC 6 mg/ml/min (4 pts; 6,1,6,3 cycles); 3 dose limiting toxicities (DLTs)
DL4: Indisulam 600 mg/m*, carboplatin AUC 5 mg/ml/min (2 pts; 1, <1 cycles); 1 DLT
Patient accrual to DL4 is still ongoing. Median 4.5 cycles (range <1–7 cycles) could be administered. Toxicity was assessed according to NCI-CTC, version 2.0. Thrombocytopenia G4 revealed to be the leading DLT (3 pts), followed by granulocytopenia G4 (1 pt). The non-hematological toxicity was minimal. The preliminary pharmacokinetic investigation let assume that carboplatin does not affect the kinetics of indisulam.
**Conclusions:** Hematotoxicity is dose limiting, necessitating dose reductions in four patients and almost always delay of retreatment by one week awaiting recovery from myelosuppression. The recommended dose for the further phase II study has not been identified yet.

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**Identification of clinically relevant pharmacodynamic biomarkers in patients treated with the CDK inhibitor CYC202 (R-roscovitine)**

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CYC202 (R-roscovitine) is an inhibitor of Cyclin Dependent Kinases (CDK), which is currently in phase II clinical trials. CYC202 is a potent inhibitor of members of the CDK family with best activity against CDK2/cyclin E (IC50=80nM), CDK2/cyclin A, CDK7/cyclin H and CDK9/cyclin T. When tumour cells are treated with CYC202 there is a slight accumulation in G2/M but ultimately CYC202 causes apoptosis in tumour cells from each phase of the cell cycle. Previously CYC202 has been shown to be active against a range of human tumour cell lines both in vitro and in vivo.
CYC202 has completed two phase I studies while three phase II trials are currently ongoing: in combination with gemcitabine/cisplatin for NSCLC; in combination with capecitabine for breast cancer; and as a single agent in haematological B-cell malignancies. Samples from these clinical studies are being used for the identification of pharmacodynamic biomarkers. Three types of biomarkers are being studied: 1) markers of biological activity; 2) markers of anti-tumour activity and 3) markers of patient response. Towards this end, a broad range of approaches are being taken including: 1) analysis of CYC202 induced changes in patient plasma proteomic profiles using SELDI-TOF-MS; 2) the use of ELISAs to monitor patient plasma for markers of CYC202 induced cell death and 3) microarray analysis to monitor the effects of CYC202 on gene expression and initial experiments aimed at identifying in vitro genetic signatures that correlate with CYC202 sensitivity or resistance.
Candidate biomarkers have been identified in patient samples from the Phase I clinical trials and these markers are now being further examined in samples from the Phase II clinical trials. Results will be presented from these continuing experiments describing the identification of pharmacodynamic biomarkers to support CYC202 clinical development.

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**A comparison of clinicopathological features and molecular markers in British and Nigerian women with breast cancer**

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**Background:** Several studies have suggested that breast cancer in black women is a more aggressive disease than in white women. This study compares the clinical stage, histological grade and expression of five molecular markers in breast cancer material from Nigeria and United Kingdom.
**Methods:** The histological diagnoses of 178 consecutive Nigerian patients with breast cancer and 113 consecutive British patients with breast cancer were retrieved from their hospital records.
A subset of 72 age-matched Nigerian and British patients was staged and their tumours typed and graded. Immunohistochemical staining of sections

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

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**The interactions of acyclic nucleotide analogues with human telomerase**

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**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.

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

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

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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.