interaction with GSH/GST pathway. Interestingly, its sequence-specificity is completely different from that of tallimustine whose DNA interaction is not affected by the presence of GSH/GST. The DNA interaction and the sequence-specificity of PNU-571077 are superimposable to that of brostallicin. These findings further support the role of GSH in the mechanism of action of brostallicin.

544 POSTER

Atomic force microscopy study of structural transitions of supercoiled DNA in response to Poly(ADP-ribose)polymerase-1 protein binding

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Background: Poly(ADP-ribose) polymerase (PARP-1) is a multifunctional nuclear DNA-binding protein that interacts with single and double stranded DNA breaks as well as with secondary structures in undamaged, supercoiled DNA. Secondary structures of DNA, such as cruciforms, can play a role in transcription by creating new protein binding sites, to which various factors bind and restrict movement of the transcription-elongation complex. Modulating the level of DNA supercoiling has been proposed as a possible mechanism for regulating gene expression at a distance. PARP-1 has been shown to affect transcriptional regulation of specific genes.
Materials and methods: Supercoiled topoisomers of the cruciform structure containing plasmid, pUC8F14C, were used as substrate recombinant human PARP-1 binding. Atomic force microscopy (AFM) images were obtained using the NanoScope IIIa instrument equipped with an E-scanner (Digital Instruments, Santa Barbara, CA) and analyzed by using the computer program accompaning the imaging module.
Results: We observed that PARP-1 binds to the ends of the hairpin arms of the topoisomers of pUC8F14C DNA. This DNA contains a 106-bp F14C inverted repeat with predicted cruciform arm length of 53 bp. Analysis of the volume distributions of PARP-1 molecules in DNA-PARP-1 complexes revealed that PARP-1 forms a few dimers on interaction with cruciform structure. We determined that when PARP-1 binds to one segment of the supercoiled plasmid DNA in these complexes, it appears partially relaxed. Whereas, when PARP-1 interacts with nodes, it makes a DNA but, the level of supercoiling in the surrounding plasmid does not decrease.
Conclusions: Proteins that bind to enhancer elements and interact with the transcriptional machinery regulate transcription. Protein binding may alter local DNA structure through the change of DNA superhelicity. In previous work, we found that PARP-1 repressed transcription when it binds to the PARP promoter. This suggests that the affinity of PARP-1 to secondary DNA structures and the changes in the topology of supercoiled DNA generated by PARP-1 binding across poly(ADP-ribosyl)ation reactions can play a role in the regulation of gene expression. Accordingly, PARP inhibitors may be therapeutic agents capable of gene regulation.

545 POSTER

Biological effects of G-Quadruplex binding agents in various human cancer cell lines

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Findings that expression of telomerase and the maintenance of telomere length in the overwhelming majority of tumours together with the absence of such features in normal somatic cells, have created much interest in targeting the enzyme and telomeres, as a new cancer drug discovery strategy. An approach developed by this laboratory involves targeting the 3′-single stranded overhang telomeric DNA substrate, by inducing it to fold into a four stranded guanine-quadruplex structure (G4) that is incompatible with telomerase extension, and which itself may serve as a signal for DNA damage responses.
A series of small molecules that have been designed and synthesised to stabilise G4 structures, have been previously reported by us. These compounds have shown inhibitory effects against telomerase, detected by the TRAP assay. Many of these compounds have demonstrated selective potency against human carcinoma cell lines in short-term cytotoxicity studies while presenting low toxicity against normal human cells. Further, an initial lead compound, the 3,6,9-trisubstituted acridine BRACO-19, has displayed long term growth arrest in carcinoma cell lines and replicative senescence in vitro as well as in vivo activity in a tumour xenograft model. These in vitro cellular effects are both dose and time dependent. The loss of chromosomal integrity by generating end-to-end chromosomal fusions produced by BRACO-19 is consistent with its rapid induction of telomere uncapping.
We report here on detailed cellular and molecular studies for a new set of 3,6,9-trisubstituted acridines, with the goal of establishing structure-activity relationships and identifying optimal candidate telomere maintenance inhibitor molecules for subsequent in vivo studies. A panel of carcinoma cell lines, representing prostate, breast, non-small-cell lung and ovarian cancers, has been established, together with a series of evaluation criteria. Possible reasons for the observed differences in responses will be presented, together with details of the structure-activity relationships. The evaluations involve comparison of quadruplex affinity, telomerase inhibition, with potency in long-term inhibition-of-proliferation studies and measurements of apoptosis and senescence.

546 POSTER

A novel aureolic acid antibiotic analogue has potent anti-proliferative activity and induces multiple changes in gene expression in ovarian cancer cells

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Aureolic acid antibiotics are interesting lead compounds for drug development because of their ability to bind to GC-rich sequences in DNA, block binding of Sp1-family transcription factors and inhibit transcription of Sp1-regulated genes preferentially. Aureolic acid antibiotics, such as mithramycin and chromomycin, are active against several types of cancer but their clinical use is limited by severe side effects. In the attempt to identify compounds with improved activity and therapeutic index, we have evaluated the activity of a new aureolic acid analogue, SDK, which had been generated by genetic manipulation of the mithramycin biosynthetic pathway in *S. Argillaceus*. SDK was an effective inhibitor of proliferation of several ovarian cancer cell lines with IC_{50} concentrations ranging between 50 and 250 nM. Flow cytometry analysis of A2780 ovarian cancer cells showed cell cycle alterations and induction of massive apoptotic cell death as indicated by the appearance of a prominent sub-G1 peak after 24 and 48 hours of drug treatment. To determine the mechanisms involved in the response of ovarian cancer cells to SDK, we evaluated its effects on gene expression after 6 hours of incubation of A2780 cells using Affymetrix U133 GeneChips. Multiple genes involved in transcription regulation, DNA repair, cell cycle, proliferation, apoptosis and angiogenesis, were n egatively modulated by SDK. Gene expression analysis by RT-PCR and Western blotting confirmed that SDK induced down-regulation of genes, such as c-myc, c-src, hTERT, Bcl-XL, Ets2 and VEGF at low concentrations (50–100 nM) and early time points (<24 hours). The ability of SDK to inhibit cell proliferation and modulate expression of critical cancer promoting genes is an important feature for further development of this compound as a cancer therapeutic agent.

547 POSTER

DNA adduct formation by C-1748, a potent antitumor 4-methyl-1-nitroacridine of lowered toxicity

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4-Substituted 1-nitroacridines represent a new group of acridine derivatives synthesized at Gdansk University of Technology. In contrast to parent 1-nitroacridines, these compounds exhibit low toxicity and enhanced antitumor efficacy. The leading derivative 4-methyl-1-nitroacridine denoted C-1748 is being prepared for phase I clinical evaluation. The introduction of an electron donating methyl group into position 4 (para to 1-nitro) decreased the susceptibility of 1-nitro substituent to reduction. 1-Nitro group is crucial for biological activity and also for the ability of 1-nitroacridines to form DNA adducts. In the present study, we investigated DNA binding properties of C-1748 in comparison to the parent 4-unsubstituted analogue – C-857. Two methods were used: the elaborate [32P]-post-labelling technique and a newly developed by us simple and rapid method exploiting restriction enzymes to the detection of covalent modification of PCR-amplified DNA fragment. The latter approach enabled us to demonstrate covalent binding of C-1748 to DNA in different activating systems and to study kinetics of this reaction. The one involving DTT as a reducing agent required long exposures of DNA to C-1748 (17 h), while C-857 modified DNA within 1 h. In the presence of microsomes, short incubation times (1–3 h) were required for both compounds. For both acridines, the level of binding was concentration- and time-dependent. Another difference revealed by this method was the base pairs preference; C-857 was clearly GC specific, while C-1748 seemed to bind with similar efficiency to both GC and AT base pairs. In parallel, the detection of DNA adducts was carried out by [32P]-post-labelling technique. The maps of [32P]-labelled adducts formed by C-1748 displayed more chromatographic

spots than those obtained for C-857 under experimental conditions. This technique was used also to demonstrate the DNA adduct formation by C-1748 in human colon carcinoma HT-29 cells. The chromatographic pattern of DNA adducts detected resembled the ones observed in cell-free system. In conclusion, current studies along with interstrand DNA crosslinking demonstrated for a number of 4-substituted analogues suggest that also this new generation of 1-nitroacridines with lowered toxicity are able to bind covalently to DNA. This implies that DNA represents their major molecular target whose covalent modification induces a cascade of biological events eventually leading to apoptosis.

548 POSTER

Oral administration of clofarabine daily × 5 every 4 weeks in patients with advanced solid tumours in a phase I and pharmacokinetic study

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Background: Clofarabine, a next-generation purine nucleoside analogue, inhibits DNA polymerase α and ribonucleotide reductase and disrupts mitochondrial integrity resulting in release of cytochrome C and apoptosis-inducing factor. Several clinical trials examined the activity of an intravenous infusion (IV) of clofarabine in solid tumors and hematological malignancies. However, the oral administration of clofarabine is also possible and may offer advantage over the IV form. For example, superior curative activity was observed with daily oral compared to IV clofarabine administration in HT-29 and colon 36 xenograft models. Therefore, a phase I study to determine the safety and appropriate dose of oral clofarabine is warranted. **Methods:** Pts with advanced solid tumors that failed conventional therapy were treated with clofarabine administered orally for 5 consecutive days every 28 days. Cohorts of pts were dose-escalated according to a modified Fibonacci scheme to determine DLT and the MTD. **Results:** To date 11 pts (M/F: 4/7; median age=64) with advanced solid tumors (kidney n=4, colon n=2, adenoid cystic n=1, bladder n=1, cervical n=1, non-small cell lung n=1, and squamous cell skin n=1) have received 29 cycles (median 3; range 1–4) of oral clofarabine over 4 dose levels (1.0, 1.5, 2.25, and 3.5 mg/m^2). Best response to date: stable disease in 7 pts; progressive disease in 3 pts; and 1 pt pending response assessment. Cycle 1 drug-related grade 1–2 toxicities include: fatigue (n=6), nausea (n=4), abdominal cramping (n=2), anemia (n=2), stomatitis (n=2), leucopenia (n=1), thrombocytopenia (n=1), emesis (n=1), pruritis (n=1), diarrhea (n=1), and myalgias (n=1). One pt with cervical cancer treated at 2.25 mg/m^2 experienced grade 3 diarrhea on cycle 1 day 2, but subsequently was removed from study on day 19 due to obstruction from a large pelvic mass requiring an ileocolostomy. Maximal plasma clofarabine concentrations in the 1, 1.5 and 2.25 mg/m^2 cohorts averaged 5.3 (±1.6), 7.6 (±4.0) and 10.3 (±4.3) ng/mL, respectively. $AUC_{(0-24)}$ averaged 40.7 (±13.9), 59.6 (±16.1), and 87.8 (±27.4) ng*h/mL. Both C_{max} and $AUC_{(0-24)}$ increased with clofarabine dose. The accumulation ratio after 5 days was 1.4 (±0.33). Based on historical IV data, the oral bioavailability of clofarabine was estimated to be >70%. **Conclusion:** Oral clofarabine shows good bioavailability with characteristics of dose-dependent absorption. Accrual continues at 3.5 mg/m^2 to further define the MTD.

549 POSTER

HKH40A, potent agent agaist GI cancers, targets p53 or when that is mutated, Akt

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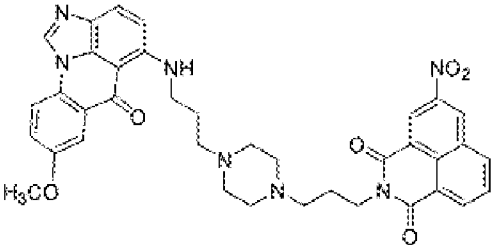
HKH40A is a synthetic agent with very potent but selective activity against gastro-intestinal cancers.

The compound binds to genomic DNA by intercalation of one of the aromatic residues, with the rest of the molecule residing in the minor groove. The operational hypothesis is that the complex "hijacks" critical proteins involved in DNA repair and transcription. Expression array studies have shown that the compound affects the expression of numerous genes in tumor cells, many of them associated with the cell cycle and apoptosis. HKH40A and its closely related des-methoxy analog WMC79 are very toxic to human colon cancer cell lines that express the wild type p53 tumor suppressor gene (LC_{50} = 25nM for RKO and HCT116 cells for HKH40A). Those cells are killed by a p53-dependant apoptotic cascade, initiated by a rapid upregulation of p53, which results in the activation of the FasL pathway, upregulation of the Bax/Bcl2 ratio and the resultant activation of the mitochondrial apoptosis pathway. All these biochemical changes result in activation of caspase 3 that in turn activates pro-apoptotic endonucleases. Upregulation of p53 is frequently a response to DNA damage, which in this case may be the consequence of HKH40A (and WMC79) being potent topoisomerase-1 poisons. However, experiments with topoisomerase-1 deficient cells showed that the enzyme is not the only target for the drugs. HKH40A is also a very potent agent against pancreatic and liver cancer cells (LC_{50} = 80 nM for ASPC-1 and 60 nM for Hep3B). The target for the drug in these tumors is not p53 since that is either mutated or not expressed. The compound arrests the growth of these cells at the G2-M checkpoint (upregulation of cyclin B1 and sustained phosphorylation of cdk1). All of these cell lines overexpress the phophorylated form of Akt, which is a pro-survival protein since it inhibits several key elements of apoptosis. HKH40A is a very potent inhibitor of phospho-Akt and the upstream PI3 kinase in pancreatic adenocarcinoma and hepatocellular carcinoma cells that we examined. The cell cycle effects are consistent with this finding. We conclude, that phospho-Akt is the molecular target for HKH40A in those cancers that express the protein (pancreas and liver). However, in wt p53 cancers the inhibition of topoisomerase 1 and the activation of the p53 cascade appear to be the principal targets for the drug. HKH40A is curative for orthotopic liver cancer in rats, with no evidence of toxicity. HKH40A is a prime agent for clinical development.



550 POSTER

Phase I and pharmacokinetic (PK) study of trabectedin (ET-743) administered as a 1-hour infusion weekly for 3 consecutive weeks every 4 weeks to patients with advanced cancer

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Trabectedin is a tetrahydroisoquinoline alkaloid isolated from *Ecteinascidia turbinata* covalently targets guanine in GC-rich region of DNA minor groove creating DNA bend towards the major groove; interferes transcription factors-DNA interaction; and causes DNA breaks by nucleotide excision repair. Thus cancer cells undergo apoptosis or cell cycle arrest. Trabectedin has been tested in phase I and II studies using different infusion schedules and doses and is well tolerated with preliminary activity in sarcoma, breast and ovarian cancer. To maximize the tolerability, efficacy, and overall therapeutic index of trabectedin, this study evaluated the feasibility, safety, and PK behavior of trabectedin as a 1-h infusion weekly ×3 every 4 weeks. The results of a previous study at our institution indicated a favorable toxicity profile and antitumor activity when the agent was administered over 3 hours weekly ×3 every 4 weeks. To date, 31 pts (median age 45, [23–75]; M:F 17:14; tumor types: sarcoma:ovarian:breast:melanoma 27:2:1:1; 105 Cycles was delivered with a median of 2 [1–14] over 6 dose levels (460 [6], 580 [3], 610 [6], 700 [8], 800 [6 lightly-pretreated (LP) & 1 heavily-pretreated (HP)], 920 mcg/m2 [1]). Dose-limiting toxicities (DLTs) during the first 2 cycles include: gr 4 ANC >5 d [1] at 460; gr 3 myalgia/fatigue [1] at 610; delay of Cycle 2 >2 wk for ANC<1,500, gr 3 neuropathy/fatigue, febrile neutropenia [1 each] at 700; gr 4 neutropenia, followed by rhabdomyolysis and death at 800 in a heavily-pretreated ovarian cancer pt with compromised bone marrow reserve due to repeated carboplatin exposure; treatment held for 2 weeks [1] at 920. Toxicities were mostly mild to moderate except: asymptomatic gr 3 transaminase elevation [22.5%], gr 3/4 CK [6%], gr 3/4 ANC [19%], gr 3 fatigue and myalgia [3%] and gr 3 vomiting [3%], which all occurred at doses ⩾700. PK evaluation up to 700 demonstrated linearity, similar to prior data of other dosing schedules, with $\tau_{1/2}$ 56.5±54.2 h and Vss 2463±1580 L. A confirmed PR was observed for 36 wk in a second-line metastatic uterine leiomyosarcoma; and SD in 4 leiomyosarcoma [24–28 wk], 2 liposarcoma [14, 56 wk] and 1 fibrosarcoma [16 wk] were observed. Clinical activity was seen in selected soft tissue sarcoma subtypes, which failed prior doxorubicin and/ifosphamide-based