Methods and Results: The maximum tolerated dose (MTD) for MTX and MTX-albumin was determined (2 mg/kg based on MTX injected on day 1, 3, and 7). 100 SD-rats bearing Walker-256 tumors received injections of the MTD and of MTD/2 of MTX, MTX-albumin and of mixtures containing MTD/2 or MTD/4 of both MTX and MTX-albumin (MTX-MIX). In addition 30 Copenhagen rats bearing a MTX resistant slowly growing Dunning Hi prostate adenocarcinoma (Du HI) were treated with the MTD of MTX and of MTX-albumin. No side effects were observed. MTX-albumin conjugates were more effective than MTX alone in terms of growth retardation of the Du Hi tumor ($p < 0.001$). In the Walker-256 tumor bearing rats, cures and growth retardation were observed with the lowest rates for MTX alone, than for MTX-albumin, and with the best results for the combination of MTX and MTX-albumin. This was confirmed for the MTD and MTD/2 group. At 1 mg/kg MTX cured 2 out of 10 rats, MTX-albumin 3 of 10, whereas a mixture 0.5 mg/kg of MTX and of 0.5 mg/kg MTX-albumin cured 6 out of 10 rats and prolonged the surviving time from 4.7 days to 7.3 days compared to MTX.

Conclusion: MTX-albumin conjugates show therapeutic activity in vivo. In combination with MTX additive effects were observed. MTX-albumin conjugates performed significantly better than the parent compound in a slow growing rodent tumor.

801 POSTER

The antihypercalcemic action of gallium-nitrate is not due to inhibition of parathormone or parathormone-related protein secretion in rats

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Purpose: Gallium nitrate (GaN), the anhydrate salt of the naturally occurring heavy metal, is able to reduce hypercalcemia of malignancy in vivo. Furthermore, GaN is able to reduce parathormone (PTH) secretion in parathyroid cells in vitro. We tested whether GaN is able to reduce PTH or parathormone-related protein (PTHrP) secretion in vivo.

Methods: We used female Fisher rats, weighing 160–200 g. Humoral hypercalcemia of malignancy was induced by subcutaneous inoculation of 10^6 Walker carcinosarcoma (WCS) 256 cells. PTH secretion was examined in normal animals, after inducing hypocalcemia with 100 mg/kg EDTA intraperitoneally.

Results: 40 mg/kg GaN led to a significant reduction of WCS tumor growth (5.1 ± 1.8 vs. 7.4 ± 2.2 g) and hypercalcemia (3.6 ± 0.5 vs. 4.3 ± 0.6 mmol/l) at day 8.40 mg/kg GaN did not influence PTHrP serum levels in WCS bearing rats at day 8 (27.8 ± 11.5 vs. 25.9 ± 6.2 pmol/l), whereas osteoclast surface (OcS/BS) was significantly reduced (3.5 ± 1.3 vs. $6.2 \pm 2.3\%$). EDTA-stimulated induction of PTH secretion in normal rats was not significantly reduced by 40 mg/kg GaN (133.8 ± 45.1 vs. 136.6 ± 66.8 pg/ml).

Conclusion: The antihypercalcemic effect of GaN is due to osteoclast inhibition and is not due to inhibition of PTHrP or PTH secretion in vivo.

802 POSTER

Stealth liposome entrapped doxorubicin (SLED) and cisplatin (SLEC) versus head and neck xenograft tumours

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Purpose: To study the effect of SLED and SLEC, compared to unentrapped doxorubicin (UD) and cisplatin (UC), in head and neck cancer xenograft tumours (HNCXT).

Materials and Methods: Groups of 8–10 nude mice with HNCXT received single i.v injections of one of the following agents: SLED, SLEC, UD or UC. Control animals received no therapy. Tumour volume was assessed on the day of treatment (Vo) and then 2–3 times per week. Mice were killed when the tumour had tripled its original volume (3Vo). Time taken to reach 3Vo was used as a surrogate measure of survival.

Results: Median times to 3Vo were as follows: 7.3 days (control); 9.3, 5.4, and 9.7 days (UD 50, 100 and 200 μg); 16.1, 18.3 and 40.6 days (SLED 50, 100 and 200 μg); 6.9 and 15.3 days (UC 100 and 250 μg); 15.9, 21.5 and 34.0 days (SLEC 100, 250 and 500 μg). Durable complete response or stable disease (>60 days) was seen after 200 μg SLED in half the mice. 500 μg UC caused the death of all animals at 5 days. No toxicity was seen with single dose SLED or SLEC.

Conclusion: SLED and SLEC show significant activity in HNCXT. Both SLED and SLEC were more active than their unentrapped counterparts. Clinical trials of both agents in patients with head and neck cancers are planned.

803 POSTER

Effects of MTA (multi-targeted antifolate, LY231514) on intracellular folate and nucleoside triphosphate pools in CCRF-CEM cells

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Purpose: MTA (LY231514) is a novel pyrrolo[2,3-d]pyrimidine-based antifolate, presently in phase II trials for various solid tumours. It has been shown to inhibit thymidylate synthase (TS), dihydrofolate reductase and glycinamide ribonucleotide formyltransferase *in vitro*. Both thymidine and hypoxanthine are required to completely reverse the cytotoxicity of MTA (at $\geq$30 nM) in CEM cells. The present study examined the effects of MTA on intracellular folate, ribo- and deoxyribo-nucleoside triphosphate (rNTP and dNTP) pools.

Methods: Intracellular folates were pre-labelled by culturing CCRF-CEM cells in medium containing ^{3}H-leucovorin. After drug treatment, the folates were extracted, treated with conjugase and analyzed by HPLC. Total NTPs were extracted in 60% ethanol. rNTPs were analyzed directly on HPLC, and dNTPs likewise after per-iodate degradation of rNTPs.

Results: Treatment with MTA (300 nM) for 4 h resulted in no detectable accumulation of dihydrofolate. Over 24 h, MTA caused little change in levels of rNTP, but induced a rapid loss of TTP, dCTP and dGTP (to <15%), with a concomitant rise in dATP (~30%).

Conclusion: Our data qualitatively resemble those reported for TS inhibitors (*Biochem Pharmacol* 1995; *49:* 819), suggesting that inhibiting the thymidylate cycle is a key effect of MTA in CCRF-CEM cells. Studies on the anti-purine effect of MTA are in progress.

804 POSTER

Clofazimine and B4121 sensitize an intrinsically resistant human colon cancer cell line to paclitaxel and taxotere

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Purpose: To investigate the potential of clofazimine and a more active derivative B4121, to sensitize three intrinsically resistant human colon cancer cell lines ($CaCo_2$, ATCC HTB 37; COLO 320DM, ATCC CCL 220; HT-29, ATCC HTB 38) to vinblastine, doxorubicin, daunorubicin, paclitaxel, taxotere and cisplatin at a non toxic, therapeutically relevant concentration of 0.25 μg/ml. Cyclosporin A (CsA) multidrug resistant (MDR)-modifying agent at 5 μg/ml was included for comparison.

Methods: Cell proliferation and P-glycoprotein (P-gp) expression were measured by colorimetric and flow cytometric procedures.

Results: The cell line expressing high levels of P-gp, COLO 320 DM, was susceptible to chemosensitization by the experimental agents for the P-gp substrates (paclitaxel, taxotere, daunorubicin, vinblastine and doxorubicin) but not for cisplatin. Clofazimine, B4121 and CsA increased the sensitivity of COLO 320 DM cells for paclitaxel 7, 30 and 47 fold and taxotere 5, 10 and 1460 fold respectively. $CaCO_2$ cells expressed low levels of P-gp and were only marginally susceptible to sensitization by these drugs whereas the HT-29, a P-gp negative cell line, was unaffected.

Conclusion: The riminophenazines might prove useful for inclusion in taxotere or paclitaxel chemotherapy of P-gp expressing colon cancers.

805 POSTER

Mapping drug distribution patterns in solid tumors: Toward conformal chemotherapy for local tumor control

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Purpose: Chemotherapeutic efficacy depends on concentration and duration of drug exposure to tumor cells. Extending our ability to map and predict local drug exposure may lead to generation of treatment algorithms such that rational conformal chemotherapy of solid tumors similar to conformal

radiotherapy can result. As a first step toward conformal chemotherapy, we have developed a 3D-imaging method to quantitatively map local drug concentrations in solid tumors.

Methods: Distribution patterns in dermal murine solid tumors (SCCVII, RIF-1, and pancreatic cancer xenografts) were examined after intratumoral injection of radiolabeled 5-FU in either an aqueous solution or a gel formulation at injection-volume to tumor-volume ratios of 1:50 to 1:3. Autoradiograms and photomicrographs of tumor cryosections were analyzed using NIH Image software to obtain isodose curves. Autoradiograms were digitally combined to reconstruct the tumor in 3D. Drug concentration in any specific location was determined by digital resectioning of the reconstructed tumor image.

Results: ^{14}C-5-FU administered in a simple aqueous solution was rapidly distributed throughout the tumor (3–19 mM, at 2 min, depending on dose volume) and cleared quickly (0.2–3.4 mM at 1 h). 14C-5-FU administered in the gel was distributed through 60–80% of the tumor at 2 min (21–111 mM) and through 80–100% of the tumor at 1–2 h (8–13 mM). Distribution pattern varied with tumor tissue and formulation. Isodose curves were concentric after administration of 5-FU gel. Concentrations depended on dose volume and decreased exponentially as a function of distance from the margin of the gel. Overall exposure (extent × concentration × time) was severalfold higher using the gel formulation than that using the solution.

Conclusion: This 3D mapping technique may enhance our ability to predict local drug exposure in solid tumors and contribute to the development of conformal chemotherapy for local tumor control.

806 POSTER

Activation of immune effector cell cytolytic activity by the alkaloid derivate Ukrain (NSC 631570)

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Purpose: To investigate the possible modulation of immune effector cells' cytolytic activities by the alkaloid thiophosphoric acid derivative Ukrain (NSC 631570).

Methods: The cytolytic activity of alloimmunized spleen lymphocytes and peritoneal macrophages (PM) from tumor bearing animals was investigated in vitro by ^{51}Cr release assays in the presence of various concentrations of Ukrain in the CML assays.

Results: The cytolytic activity of freshly isolated spleen lymphocytes from P815 (H-2) alloimmunized C57B1/6 mice, which had no significant endogenous cytolytic activity, i.e. 2.0% specific ^{51}Cr release, were found to increase their lytic activity to 65% in the presence of 1.2 M of Ukrain. The in vitro CML investigations were carried out at E/T = 5:1 for 3.5 hrs. The effects of Ukrain on the cytolytic activity of PM of Babl/c bearing syngeneic D1-DMBA-3 tumors were also assayed in vitro. Results showed that Ukrain at 2.5 M activated the cytolytic activity of macrophages from 0% to 13% specific lysis of syngeneic tumor cells. Moreover, in vivo studies with Balb/c mice bearing syngeneic mammary adenocarcinomas showed that Ukrain had a significant inhibition of tumor growth and progression.

Conclusions: Ukrain was found to activate the cytolytic activity of spleen lymphocytes as well as anergic macrophages obtained from mice bearing syngeneic tumors. It indicates that this compound functions as a biologic response modifier (BRM). This conclusion is further supported by the finding that Ukrain reduced significantly the growth rate of established mammary adenocarcinomas without any observable side effects.

807 POSTER

Schedule-dependent myelotoxicity induced in vitro by the new marine derived minor groove interacting agent ecteinascidin 743

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Purpose: To evaluate the toxicity on human hematopoietic progenitor cells of the marine derived anticancer compound ecteinascidin 743 (ET 743) at different schedules of exposure.

Methods: Human umbilical cord blood derived progenitors were incubated with ET 743 for 1 h, 24 h and 1 h daily × 5, then plated in clonogenic assays. The growth of erythroid (CFC-E and BFU-E) and myeloid colonies (GM-CFC) was scored after 7 days (more differentiated progenitors) and 14 days (earlier progenitors).

Results: The concentration of drug inhibiting the growth of 70% of the colonies (ID70) for each schedule was (median and range):

	ID70 (ng/ml)		
	1 hour	24 hours	1 hour daily × 5
GM-CFC d7	6 (1–13)	0.8 (0.3–3.2)	1.6 (1.2–2.0)
GM-CFC d14	12 (7.5–13)	2.5 (1–9)	3.0 (2.2–3.6)
CFC-E d7	5 (2.5–12)	1 (0.5–3.3)	2.2 (1–2.5)
BFU-E d14	9.5 (4–12.5)	1.4 (0.5–9)	2.4 (1.5–2.6)

Conclusions: Erythroid and myeloid progenitors are equally sensitive to the drug, while early progenitors are more resistant than the differentiated ones. The 1 h daily × 5 treatment is 3–4 times more toxic than 1 h exposure, suggesting that the toxicity might be schedule dependent, while the 24 hours exposure seems to be less myelotoxic.

808 POSTER

Tumor uptake of MTX-albumin conjugates in rats

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Following our observation that albumin turnover in rodent tumors is markedly increased, we present evidence that albumin can be employed for targeting methotrexate (MTX) into tumors. The discrepancy in the molecular weight of MTX (454 Da) and albumin (67000 Da) tempted to load multiple drug molecules on one carrier molecule.

Methods and Results: We will show that only a loading rate of approx. 1 mol of the cytostatic drug MTX per mol of albumin offers optimal conditions for targeting residualizingly radiolabeled methotrexate-albumin conjugates into rodent tumors (W-256 carcinosarcoma). Conjugates bearing 5, 7, 10, and 20 molecules of MTX showed signs for albumin denaturation. These conjugates, tagged with a residualizing radiolabel, were efficiently trapped by the liver before reaching the tumor tissue. Competition experiments with maleylated bovine serum albumin and fucoidan revealed that the group of scavenger receptors present on the cells of the liver monocyte macrophage system were involved in this process.

Conclusion: We chose the MTX-albumin conjugate, derivatized only at a molar ratio of approx. 1:1, for further preclinical and clinical studies. This conjugate showed the best tumor targeting properties, low liver uptake rates, and a very long biological half life.

809 POSTER

High effectiveness of combined treatments of paclitaxel (TX) and 4′-epi-doxorubicin (EP) in a murine tumour: A preclinical study

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Purpose: The optimization of schedule of combined TX and EP administration on the growth of a murine mammary carcinoma was studied.

Methods: For the experiments tumour was transplanted into the right hind foot of female hybrid (C3D2F1) mice. Drugs were delivered i.p. TX was administered in single doses from 15 to 75 mg/kg b.w. and EP was administered from 9 to 24 mg/kg b.w.. Results were analyzed in terms of Tumour Growth Delay (TGD).

Results: TGD's, in the combined administration, show an effect at least additive in all the tested protocols. Quasi-simultaneous delivery has shown at the higher doses a non tolerable toxicity, that is acceptable with a time interval of 24 h between treatments. The best result, both in terms of effectiveness and tolerability, was obtained delivering TX 45 mg/kg 24 h before EP 15 mg/kg (with a TGD of 22.6 days). The superadditive effect clearly shown by divergent linear regression curves was also obtained after quasi simultaneous treatment TX in different doses and EP 9 mg/kg.

Conclusion: The performed experiments have shown the high effectiveness of the combination of the two drugs and the importance of the delivery protocols.