grade 4 neutropenia in cycle 1. Nadir occurred at 14 days (10−16) and median time to recovery was 5 days (1−8). Nonhematological toxicity was mild/moderate and reversible: fatigue (82%), nausea (50%), diarrhea (18%) and transaminase increase (50%). There was marked interindividual pharmacokinetic (PK) variability (clearance 11.0±5.5 L/hr), with a median terminal half-life at the RD of 58 hr. There was no significant association of dose or BSA with clearance, and there was a stronger correlation of AUC than dose (mg/m^2) with neutropenia (vs. log ANC, r = −0.92 vs. −0.72).
Conclusions: PM01183 can be safely administered at the RD of 7.0 mg (>200 times the starting dose), although intrapatient dose escalation may be warranted given the magnitude of interindividual PK variability and its association with neutropenia. Cohort expansion is ongoing at the RD to better define PK/pharmacodynamic relationships, and to screen for antitumor activity.

435 POSTER

Endoplasmic reticulum stress mediates immunogenic cancer cell death via the phosphoinositide 3-kinase pathway

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Background: In response to some specific chemotherapeutic agents or ionizing irradiation, dying tumor cells can elicit a potent anticancer immune response. However, the exact mechanism determining cancer cells undergoing immunogenic cell death remains unclear. Here we explore the intracellular signaling pathway underlying wogonin-induced immunogenic gastric cancer cell death.
Materials and Methods: Two-dimensional (2D) electrophoreses, followed by mass spectroscopic analyses was used to identify the proteins regulated by wogonin in MNK-45 human gastric cancer cells. Western blots and confocal immunofluorescence were applied to examine the expression and intracellular location of proteins. Immunopercipitation and small interfering RNA (siRNA) knockdown studies were designed to determine the interaction of p22 and CRT.
Results: Wogonin induces Reactive Oxygen Species (ROS) production elicits an endoplasmic reticulum (ER) stress response, including the phosphorylation of potein kinase-like endoplasmic reticulum kinase (PERK)/protein kinase R (PKR) and eukaryotic initiation factor 2α (eIF2α). They serve as upstream signal for the phosphoinositide 3-kinase pathway activation, which induces calreticulin (CRT)/Annexin A1 cell membrance translocation and high-mobility group box 1 protein (HMGB1) release. Interestingly, a Ca^{2+}-binding protein P22/CHP associates with CRT, but not Annexin A1, and mediates its translocation to cell membrane. The releases of HMGB1 from wogonin treated MFC cells, alone or together with other factors, activates dendritic cells and induces cytokine releases. In vivo study confirms that wogonin can elicit immunogenic gastric cell death and a possible inflammatory response is involved.
Conclusions: The activation of the phosphoinositide 3-kinase pathway elicited by ROS induced ER stress causes CRT/Annexin A1 translocation and HMGB1 release, mediating wogonin-induced immunogenic gastric cancer cell death.

436 POSTER

NBRI16716A, a new antitumor compound against prostate cancer cells, produced by Perisporiopsis melioloides Mer-f16716

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Growing evidence supports the idea that the stroma in tumor tissues can regulate the tumor growth and metastasis. We focused on such tumor–stromal cell interactions of prostate cancer and reported that prostate stromal cells (PrSC) promote the growth of human prostate cancer cells through secretion of insulin-like growth factor-I (Cancer Res 66, 4419, 2006). Because small molecules that modulate the tumor–stromal cell interactions possibly show potent antitumor effect, we developed the in vitro coculture system of human prostate cancer cells and PrSC, in which the growth of prostate cancer cells is increased by the coculture with PrSC (Anticancer Res 24, 1561, 2004). Using this screening system we have been finding several natural compounds that exert antitumor effect through the modulation of the tumor–stromal cell interactions (J Antibiot 62, 243, 2009; Int J Cancer 126, 810, 2010). By further screening, we have found new modulators. Here we report about the biological activities of newly isolated three natural compounds, NBRI16716A (1), NBRI16716B (2), and NBRI16716C (3) from the fermentation broth of *Perisporiopsis melioloides* Mer-f16716. Compounds 1 and 2, but not 3, inhibited the growth of human prostate cancer DU-145 cells in the coculture with human prostate stromal cells (PrSC) more strongly than that of DU-145 cells alone. Compounds 1 and 2 did not exhibit acute toxicity in mice up to 100 mg/kg. Furthermore, both compounds showed anti-tumor effect against xenograft models of DU-145 cells and PrSC *in vivo*.
Collaborator: Mercian Corporation.

437 POSTER

The synergistic antitumorigenic effects of vinblastine and total Astragalus saponins (AST) with reduced invasiveness of colon cancer cells

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Introduction: We have demonstrated in our ongoing studies that the total saponins of *Astragalus membranaceus* (AST) possess potential proapoptotic and antiproliferative effects in human colon cancer cells and tumor xenograft via distinctive molecular pathways. The therapeutic effects of the combined use of AST and the microtubule inhibitor vinblastine (VBL) in colorectal cancer cells were investigated in the present study, with emphasis on their anti-invasive potential.
Methods and materials: MMT viability test, flow cytometry, Western immunoblotting, immunohistochemical assessment, Cell invasion assay had been used.
Results: Combined drug treatment induced a further reduction in HCT 116 cell viability when compared to either AST (80 μg/ml) or VBL (2.5 nM) treatment alone. Cell cycle distribution analysis showed that larger proportion of AST-VBL treated cells appeared to be accumulated at the G2/M phase when compared to those treated with AST or VBL alone for 24 h. Expression of both pro-caspase 3 and pro-caspase 9 were further downregulated in the combined treatment when compared to AST or VBL treatment alone for 72 h, with complementary observations in PARP cleavage. Further reduction in the protein expression of bFGF, VEGF, MMP-2 and MMP-9 was also observed with combined treatment of AST and VBL. In a HCT 116-xenografted nude mice model, combined treatment of AST and VBL showed further inhibition of VEGF expression and secretion level in the tumor tissues when compared with those in the AST or VBL alone treatment groups. One of the major side effects of VBL is to drastically weaken the immune responses of the patients by reducing the number of white blood cells (WBC). Our results show that VBL treatment alone significantly reduced the white blood cell count in Balb/c mice, but co-treatment of AST and VBL significantly restored it. Furthermore, the anti-invasion effect of AST was demonstrated using LoVo metastatic colon cancer cells. The number of invaded cells through the matrigel membrane was decreased by AST treatment. AST also increased the localization of cadherin-catenin complex at the cell membrane, indicating that AST could hinder cell invasion by modulating cell-cell interaction.
Conclusion: The synergistic anti-tumorigenic action of AST and vinblastine provides the implication of adjuvant chemotherapy, which together expands the anticancer spectrum to metastatic and advanced cancer types with reduced side effects of the latter drug.

438 POSTER

Imidazotetrainone prodrugs (temozolomide analogues) with activity independent of mismatch repair and alkyltransferase

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The imidazotetrazine ring possesses valuable pharmaceutical properties such as acid stability, oral availability, CNS penetration and even tumour-localisation. Yet, despite its blockbuster status, temozolomide remains the only drug in its class. Reasons for this may lie in the constraints on temozolomide activity imposed by its dependence on DNA mismatch repair for activity, and ready reversal of its DNA modification by alkyltransferase (MGMT)-mediated repair. Both factors limit the range of tumours able to respond to temozolomide treatment.
Herein we report the design, synthesis and preliminary evaluation of a new generation imidazotetrazines able to tame the latent, reactive alkyldiazonium intermediates released by the tetrazine ring, for therapeutic benefit. Compounds were tested (5 day exposure) in theA2780 ovarian carcinoma cell line and its cisplatin-resistant (MMR$^-$) cp70 variant. MGMT activity was inhibited by concurrent exposure to 10 mM PaTrin2.
Temozolomide showed a >30-fold dependence on MGMT and 27-fold on MMR. The equivalent ratios were 1.6 and 2.8 for DP86; 0.5 and 5.4 for DP68. Showing that the new compounds are completely independent of MGMT resistance and have a greatly-reduced dependence on MMR for activity. Four compounds have been evaluated in the full NCI 60-cell line panel where independence of MMR and MGMT was confirmed.