survivors. In contrast, 42% and 5% of the non-long-term survivors had intermediate and poor risk features compared with 28% and 0% of long-term survivors, respectively. Additional characteristics associated with LT-OS will be presented.

Variable	PFS		OS	
	HR (95% CI)	P-value*	HR (95% CI)	P-value*
Ethnic origin (white vs non-white)	0.598 (0.459, 0.781)	0.0002	0.730 (0.535, 0.996)	0.0474
ECOG PS† (⩾1 vs 0)	1.250 (1.043, 1.498)	0.0159	1.505 (1.218, 1.859)	0.0002
Time from diagnosis to treatment† (⩾1 vs <1 year)	0.814 (0.680, 0.975)	0.0252	0.666 (0.541, 0.820)	0.0001
Bone metastases (yes vs no)	–	–	1.535 (1.250, 1.886)	<0.0001
Baseline hemoglobin† (⩽LLN vs >LLN)	1.384 (1.144, 1.675)	0.0008	1.548 (1.245, 1.925)	<0.0001
Baseline lactate dehydrogenase† (>1.5 × ULN vs ⩽1.5 × ULN)	1.664 (1.201, 2.305)	0.0022	1.571 (1.103, 2.238)	0.0123
Baseline corrected calcium† (>10 vs ⩽10 mg/dL)	1.374 (1.080, 1.747)	0.0096	2.208 (1.722, 2.832)	<0.0001
Baseline neutrophils (⩽ULN vs >ULN)	0.629 (0.483, 0.821)	0.0006	0.681 (0.508, 0.915)	0.0107
Baseline platelets (⩽ULN vs >ULN)	0.607 (0.469, 0.785)	0.0001	0.670 (0.505, 0.889)	0.0055
Prior cytokine (yes vs no)	1.342 (1.085, 1.659)	0.0066	1.387 (1.094, 1.759)	0.0068

*Wald Chi-Square Test; †Factor included in MSKCC prognostic model

Conclusions: These analyses validated use of clinical risk factors previously reported from MSKCC (J Clin Oncol 20: 286, 2002) and by Heng et al (J Clin Oncol 27: 5794, 2009). These factors were predictive for shorter PFS as well. In addition, pts with bone metastases had shorter OS to sunitinib. Favorable MSKCC risk status was associated with higher likelihood of achieving LT-OS. Continued progress requires incorporation of RCC tumour-specific biology.

1007 ORAL

Efficacy of DNA Vaccination Against Anaplastic Lymphoma Kinase (ALK) in Non Small Cell Lung Carcinoma (NSCLC)

C. Voena[1], C. Mastini[1], M. Menotti[1], F. Di Giacomo[1], D.L. Longo[2], C. Martinengo[1], S. Aime[2], G. Inghirami[1], R. Chiarle[1]. [1]*Università Degli Studi, Department of Biomedical Sciences and Human Oncology, Torino,* [2]*Università Degli Studi, Department of Chemistry IFM, Torino, Italy*

Background: Lung cancer is the leading cause of cancer-related mortality worldwide. Recently, NSCLC harbouring ALK translocations have been described. Although standard chemotherapy or molecularly targeted therapies are effective in NSCLC, tumour recurrence and metastatic dissemination still remain a frequent event. Our previous findings show that ALK is an effective oncoantigen for ALK positive lymphoma vaccination and thus it could, as well, represent a feasible target for ALK positive NSCLC therapy.
Materials and Methods: We generated transgenic (Tg) mice ectopically expressing human TFG- or EML4-ALK protein in lung epithelium under the murine lung specific SP-C promoter. For DNA vaccination, we injected 50 ug of plasmid DNA in the femoral muscle of anesthetized mice for a total of at least 3 immunization, as previously described (*Chiarle et al., Nature Medicine 2008*). To evaluate the generation of an immune response, we performed an *in vivo* citotoxicity assay with CSFE-labelled cells one month after vaccination. Histology and immunohistochemistry were performed on different specimens. Tumour growth and progression was monitored overtime by Nuclear Magnetic Resonance (NMR).
Results: ALK Tg mice developed multifocal adenocarcinomas similar to human tumours, starting from 1 month after birth. A strong ALK specific CTL response was elicited in ALK positive vaccinated mice, thus demonstrating that ALK vaccination could overcome the immune tolerance to the ALK protein. By MRI analysis, vaccinated mice showed a reduced number of neoplastic foci and a smaller tumour mass as compared to mice vaccinated with a mock plasmid. The efficacy of DNA vaccination was dependent on mice age as the specific CTL activity against ALK and the ability to limit the tumour expansion decreased proportionally to the mice age. The number of T lymphocytes infiltrating both the tumours and the spared lung was significantly increased in vaccinated mice.
Conclusions: Our Tg mice represent a suitable model to dissect the role of ALK in lung tumour pathogenesis and for the development of innovative treatment strategies. Our findings indicate that ALK-DNA vaccination is able to elicit a specific cytotoxic response and to delay tumour progression in ALK+ Tg mice. Therefore, in ALK positive NSCLC a strategy that combines DNA vaccination combined with standard chemotherapy or specific ALK inhibitors could represent an alternative treatment to prevent tumour relapse or metastasis.

Poster Presentations (Sat, 24 Sep, 14:00–16:30)
Basic Science

1008 POSTER

The Effects of Telomerase Inhibitor GRN163L(Imetelstat) on Cell Cytoskeleton, Cell Cycle and Matrix Metalloproteinases

I. Mender[1], Z. Dikmen[1], S. Gryaznov[2], D. Kletsas[3], K. Erbil[4]. [1]*Hacettepe University, Clinical Biochemistry, Ankara, Turkey;* [2]*Geron Corporation, Clinical Biochemistry, California, USA;* [3]*Biology, Cell Proliferation and Ageing, Athens, Greece;* [4]*Gulhane School of Medicine, Clinical Chemistry, Ankara, Turkey*

Background: As telomerase activity can not be determined in somatic tissues but can be determined in 90% of human tumours, it is an attractive target for cancer therapy to telomerase. GRN163L is an N3′ → P5′-*thio-phosphoramidate* oligonucleotite which is complementary to the template region of telomerase RNA. We have previously reported that A549-luc cells treated before cell attachment with a single dose of GRN163L weakly attached to the substrate and remained rounded, whereas control cells exhibited typical epitheloid appearance and adhesion properties. In this study, we aimed to determine whether cell cytockeleton and adhesion proteins are relative with rapid morphologic alterations and loss of adhesion in GRN163L treated A549 cells. In addition, we investigated the potential decrease in MMP levels in GRN163L treated cells and also performed cell cycle analyses.
Material and Methods: A549 cells were plated in the presence of GRN163L (1 mM) and incubated for 24hrs. The untreated control cells and treated cells were collected following 24 hr of GRN163L incubation, then actin, tubulin and e-cadherin expressions were analysed by both Western Blot and immunohistochemistry. Real-Time PCR assay was used for cell cycle analyses and determination of MMP mRNA expressions of A549 lung cancer cells treated with GRN163L.
Results: We observed that actin, tubulin and e-cadherin expressions of GRN163L treated cells were significantly decreased within 24 hrs compared with the untreated control cells. Immunohistochemistry results also showed that all the actin and tubulin filaments were displaced and concentrated along the cell membrane. Interestingly, all of the effects were reversible after 72 hrs due to the the cessation of treatment. Additionally, according to Real-time PCR results, it was obvious that Cdk 6, cdk 4 and cyclin D1 mRNA levels that regulate the G1 phase of the cell cycle decreased following 1 week of GRN163L treatment when compared with the controls. Besides these results, MMP-2 expression of A549 cancer cells decreased following 24hrs of GRN163L treatment, but there was no significant change in MMP1 expression.
Conclusions: We can conclude that the morphological changes in cell cytoskeleton and loss of adhesion occur which ocur within 24 hr in GRN163L treated cells. These target-off effects besides telomerase inhibition decrease the adhesion, proliferation and metastatic potential of A549 cancer cells. For this reason, it may be possible to inhibit metastasis of residuel cancer cells by combining GRN163L with other chemotherapeutics or surgery.

1009 POSTER

Mammary Gland Tumour Formation in Conditional Transgenic Mice Expressing GLI1

J.H. Norum[1], M. Kasper[1], V. Jaks[1], R. Toftgård[1]. [1]*Karolinska Institutet, Department of Biosciences and Nutrition, Stockholm, Sweden*

Background: Up regulation of the Hedgehog pathway effector GLI1 in breast cancer correlates with unfavourable overall survival. The Hedgehog pathway has a role in the regulation and maintenance of CD44 positive breast cancer stem cells. Skin and intestinal stem cells express the orphan G protein coupled receptor (GPCR) LGR5. Previously, we have shown that multiparous conditional transgenic mice (MMTVrtTA;TREGLI1) expressing GLI1 develop hyperplastic lesions and tumours.
Materials and Methods: GLI1 expression was induced in female transgenic mice expressing GLI1 in the mammary gland. The mice were monitored for the occurrence of tumours. Palpable tumours and hyperplastic lesions developed in the mice with induced GLI1 expression. Normal and tumour tissue were analysed.
Results: We show that the cells of the basal cell layer of the large mammary ducts are Lgr5 positive. Lgr5 is also expressed in mammary gland tumours induced in conditionally transgenic mice expressing GLI1 in the mammary gland. Hyperplastic lesions and palpable mammary gland tumours also develop in nulliparous transgenic mice, after long term low level GLI1 expression. Both solid and acinar adenocarcinomas develop in GLI1 expressing nulliparous mice, even within the same mammary gland. The expression of the stem cell marker CD44 is increased in the mammary ducts as well as the tumours in the GLI1 expressing mice. The GLI1

induced mammary gland tumours are cytokeratin 5 (K5) and cytokeratin 6 (K6) positive.
Conclusions: Taken together these data indicate that long term low level expression of GLI1 induces formation of mammary gland tumours with a basal character and that GLI1 expression affects the mammary gland stem cells. The orphan GPCR Lgr5 is expressed in the basal cell layer of the large mammary ducts and might include the mammary stem cell population.

1010 POSTER

Vitamin D Analogs Improve the Antitumour Activity of 5-fluorouracil in Colon Cancer Model MC38

M. Milczarek[1], A. Mieczkowska[1], A. Kutner[2], J. Wietrzyk[1]. [1]*Ludwik Hirszfeld Institute of Immunology and Experimental Therapy Polish Academy of Sciences, Department of Experimental Oncology, Wroclaw,* [2]*Pharmaceutical Research Institute, PRI, Warsaw, Poland*

Background: Colorectal cancer is the third leading cause of cancer death in the Western word. Epidemiological studies strongly suggest a protective effect of calcitriol (1.25-dihydroxyvitamin D3) against colon neoplasia. Moreover, the experimental research reveals its anticancer properties against this type of cancer. The antitumour activity is observed only in hyper-physiological doses of calcitriol, which can lead to hypercalcemia. For this reason the synthesis of vitamin D analogs has been started in order to obtain compounds with better therapeutic activity. On the basis of previous studies we selected two analogs: PRI-2191 (tacalcitol, 1, 24-$(OH)_2D_3$) and PRI-2205 (5,6-trans calcipotriol), which reveal higher antitumour and lower calcemic activity as well as lower toxicity than calcitriol [1, 2, 3].
Materials and Methods: In the current work, it is presented the influence of vitamin D analogs (coded PRI-2191 and PRI-2205) on antitumour activity of 5-fluorouracil (5-FU) in mice bearing transplantable murine colon cancer MC38. The antitumour effect of combined treatment was evaluated as tumour growth inhibition (TGI), increase in life span of treated mice over control (ILS) or tumour growth delay (TGD). The monitored parameters were body weight and tumour volume, which was calculated using the formula (a^2 x b)/2, where a = shorter tumour diameter in mm and b = longer tumour diameter in mm.
Results: We evaluated the most effective dose and treatment schedule with vitamin D analogs combined with 5-FU simultaneously. These studies revealed that the most effective dose for PRI-2191 is 1 μg/kg/day and for PRI-2205 10 μg/kg/day. The best results were observed, when the analogs were injected subcutaneously, three times a week. The application of PRI-2191 or PRI-2205 improve therapeutic effect of 5-FU. Analysis of TGI and ILS indicated synergy between both compounds. Next, we examined potential ability of vitamin D analogs to prolong 5-FU's activity. In this case the application of analogs was started after ended administration of 5-FU. We observed that both analogs also in such schedule of treatment, delayed tumour growth and prolonged survival time in comparison with cytostatic given alone.
Conclusion: Both vitamin D analogs improve antitumour activity of 5-FU in the colon cancer model. We could conclude that the combined therapy of these analogs and 5-FU might be potentially applied to the clinical use.
This work was supported by Ministry of Science and Higher Education Grants: No. PBZ-MNiI-1/1/2005 "New drugs with specific therapeutic and social values" Task: "Vitamin D Analog (PRI-2191) in combination with anticancer agents. In vitro and in vivo" period of 2006–2009 and No. N N401 014535 "Supporting anticancer therapy of colon cancer by using new vitamin D analogs" period of 2008–2011

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1011 POSTER

Survival of Mice With Erhlich Ascitic Tumour Treated With Ultra-dilutions

H. Moreira[1], M. Amorim[2], C. Maruyama[1], R. Trinca[1], C. Torres[3], R. Ornellas[4], C. Santos[5], M. Alves Júnior[6], E. Guiguer[7], B. Lira[8]. [1]*Faculdade de Medicina de Marilia, Physiology, Marilia,* [2]*Universidade Federal do Rio de Janeiro, Collective Health, Rio de Janeiro,* [3]*Universidade de Marília, Pharmacology, Marilia,* [4]*Universidade de São Paulo, Family Health, São Paulo,* [5]*Faculdade de Medicina de Marilia, Hemocenter, Marilia,* [6]*Faculdade de Medicina de Marilia, Experimental Surgery, Marilia,* [7]*Universidade de Marilia, Pharmacology, Marilia,* [8]*Instituto FAO do Brasil, Science, Rio de Janeiro, Brazil*

Background: This study evaluated the effectiveness of ultra-dilutions (homeopathic remedies on the scales: Hahnemannian decimal − DH and fifty milesimal − LM) on the survival of animals inoculated with Ehrlich ascitic tumour which is highly aggressive and lethal, as a possible therapy for this disease.
Material and Methods: Forty male Swiss mice, weighing about 28 grams each were inoculated intraperitoneally with 10^3 viable cells of Erhlich ascitic tumour. The animals were divided into four groups randomized of ten each (A, B, C and D). Group A was the untreated control. Animals from other groups received as treatment ultra-diluted homeopathic remedies as FAO (Factors of Self Organization) complex in a blind study: *Antimonium crudum*, *Kali carbonicum*, *Mercurius solubis*, *Sulphur*, *Natrum Muriaticum*, *Aurum metallicum*, *Ammonium Muriaticum*. The ultra-dilutions indicated for groups were as follows: B − 12DH/9DH − 5 hours after 10DH/9DH; C − 11DH/9DH − 5 hours after 10DH/9DH; D − 4LM/2LM − 5 hours after 3LM/2LM. The animals were observed until their death, about the survival time in days. The project was approved by the research ethics committees of the University of Medicine of Marília with Protocol 655/08 and followed the guidelines of the Committee on Care and Use of Laboratory Animal Resources, National Research Council, U.S.A.
Results: In group A, the first death occurred at 18 days and the last at 40 days. All mice had ascites and cachexia. In group B, the first death occurred at 75 days. In group C, the first death occurred at 299 days. In group D, the first death occurred at 146 days. After 570 days from experiment beginning, there were still animals alive and in good general condition, presenting the following percentages of survivors: B (10%), C (50%) and D (50%). These animals were euthanized in a CO_2 (carbon dioxide) chamber followed by a macroscopic necropsy. Results showed the absence of ascites and presence of congestion in organs of these animals, such as liver, spleen and lung. The number of survivors was analyzed by Fisher's exact test, comparing groups of animals treated with the control group. The results demonstrated significant differences between the control group A and treated groups C and D, considering the results of probability of $p < 0.05$.
Conclusion: The ultra-diluted remedies used as FAO complex were effective against Ehrlich ascitic tumour. Animals treated had a survival at least 14 folds greater compared to the control group. This study demonstrates the possibility of using ultra-diluted remedies in the treatment of cancer, requiring further studies to exploit these impressive results.

1012 POSTER

Activation of Nuclear Factor Kappa B and Induction of Migrationinhibitory Factor in Tumours by Surgical Stress of Laparotomy Versus Carbon Dioxide Pneumoperitoneum in a Nude Mice Model

A. Tawfik Amin[1], S. Kitano[2], N. Shiraishi[2], S. Ninomiya[2], M. Tajima[2], M. Inomata[2]. [1]*South Egypt Cancer Institute, Surgery Department, Assiout, Egypt;* [2]*Oita University Faculty of Medicine, Gastroenterology Surgery Department, Oita, Japan*

Background: Surgery is the most effective method for the treatment of malignant tumours. However, surgical trauma seems to be associated with enhanced incidence of tumour growth and establishment. At the same time, the mechanisms by which surgical trauma may have an impact on tumour growth and progression still are unclear. Laparoscopic surgery, accepted as a minimally invasive procedure, recently has been adapted for gastrointestinal cancers. Although few clinical studies have shown the oncologic feasibility of laparoscopic surgery, several animal studies mostly have shown that laparoscopic procedures are associated with significantly less increase in tumour growth and metastasis than open surgery. However, a more precise conception regarding the ability of laparoscopic techniques to treat malignant tumours still is needed. A few animal and clinical studies have evaluated the induction of adhesion molecules, inflammatory response, cytokines, and growth factors such as TNFa and vascular endothelial growth factor (VEGF) after laparoscopic and open surgery. These factors may act as indicators for the extent of surgical stress and may modify the biologic activity of dormant cancer cells after surgery. However, these factors have not been evaluated in the tumours after surgery. The authors studied the effect of carbon dioxide (CO2) pneumoperitoneum versus laparotomy on tumour necrosis factor-a (TNFa), migration inhibitory factor (MIF) expression, and nuclear factor kappa B (NFkB) activity in human gastric cancer.
Methods: Nude mice were inoculated intraperitoneally with human gastric cancer cells (MKN45). Then laparotomy, CO_2 pneumoperitoneum, and anesthesia alone were performed randomly. Tumour growth and associated TNFa and MIF expression and NFkB activity were determined.
Results: Total tumour weight, especially at the anterior abdominal wall, was higher after laparotomy than after CO_2 pneumoperitoneum ($p < 0.05$). The mRNA expression of TNFa was higher 24 and 48 h after laparotomy than after CO_2 pneumoperitoneum ($p < 0.05$ and $p < 0.01$, respectively). At all the examined time points, MIF mRNA expression also was higher after laparotomy than after CO_2 pneumoperitoneum ($p < 0.05$ until 1 week or