administration. RGTA-OTR4120 did not have an effect on amylase or total protein secretion.
**Conclusion:** RGTA-OTR4120 administration has a positive effect on salivary flow rates in irradiated mice on the short term. The effect was absent 10 weeks after radiotherapy, while at that time point, mucin producing activity of acinar cells was elevated by RGTA-OTR4120 administration. Given these results and the advantages of RGTA use in irradiated patients, further investigation on the potential of this drug to treat radiation-induced xerostomia, alone or in combination with other drugs, such as amifostine, is suggested.

**1084** POSTER

**Claudin-1 Acts as a Tumour Suppressor in Hepatoma Cell Lines**

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**Background:** Altered expression of tight junction proteins such as occludin and claudins is widely implicated in carcinogenesis. In our previous study, we detected an overexpression of claudin-1 in a subset of HCCs. Furthermore, in an immunohistochemical study by Higashi et al., correlation was found between decreased claudin-1 expression and dedifferentiation, as well as portal invasion of HCCs. Our aim was to investigate how claudin-1 influences cell proliferation in hepatoma cell lines and to determine whether it acts as a tumour suppressor.
**Methods:** To establish a cell line that stably overexpresses claudin-1, low-expressing HepG2 cells were transfected with pCI-neo vector containing full-length claudin 1 cDNA and selected with geneticin. The control line was transfected with empty pCI-neo vector. Downregulation of claudin-1 expression was carried out by siRNA targeted to claudin-1 in Hep3B cells that exhibit high basal claudin-1 levels. Down- and upregulation of claudin-1 expression was confirmed by quantitative RT-PCR and Western blotting. Cell proliferation was investigated with sulforhodamine-B test. HepG2 cells (20 million/animal) were injected subcutaneously into nude mice (5−5 for CLDN1-overexpressing and control cells) in order to investigate the tumour formation.
**Results:** Stably transfected HepG2 cells showed an overexpression of claudin-1 (10-fold at mRNA, and 2-fold at protein level) as compared with control cells. On the other hand, siRNAs decreased claudin-1 expression by 67%. According to the sulforhodamine-B test, downregulation of claudin-1 expression resulted in an accelerated cell proliferation of Hep3B cells (1.35-fold; $p < 0.01$), whereas increased claudin-1 production minimally decreased the proliferation index of HepG2 cells (0.97-fold; $p < 0.01$). Tumour formation of HepG2 controll cells was observed in 3/5 mice, whereas no subcutan nodules could be detected in animals with HepG2-CLDN1 cells ten weeks after the injection.
**Conclusions:** The minimally reduced cell proliferation together with the inhibited tumour formation of HepG2 cells due to claudin-1 overexpression, as well as the accelerated cell division by siRNA silencing in Hep3B cells indicate that claudin-1 acts as tumour suppressor in HepG2 an Hep3B hepatoma cell lines.
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**1085** POSTER

**The Longitudinal Trajectory of Post-Traumatic Growth: a Longitudinal Study**

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**Background:** The aim of the study is to investigate longitudinally the trajectory of post-traumatic growth (PTG; Tedeschi & Calhoun, 2006) in cancer patients. Secondly the study aims to study the relationship between PTG and intrusion vs. avoidance symptoms. Recently, empirical evidence highlights the presence of PTG (i.e. changes in the perceptions of oneself, one's social relationships, and one's life priorities) in 50% to 90% of patients. However less is known about the temporally trajectory of PTG and its correlates, because off the cross-sectional design of studies.
**Material and Methods:** A longitudinal study was conducted with a group of 53 cancer patients currently in the treatment and management phase of their illness. Data were collected by means of a written questionnaire, at two time points (T1 and T2) that were 24 months apart.
Post-traumatic growth was assessed by the Post-traumatic Growth Inventory. Intrusion and avoidance symptoms was measured by the Impact of Events Scale.
**Results:** Analysis showed that neither PTG levels neither avoidance symptoms change during the 24 months. On the contrary, intrusion symptoms increased significantly ($t = -2{,}02$, $df = 52$, $p < 0.05$). Further, both intrusion and avoidance symptoms was strongly related with PTG at t2 (respectively, $r = 0.37$; $r = 0.47$).
**Conclusions:** Data highlighted the temporal stability of the growth process that seems to be related to a cognitive engagement processes. From a clinical point of view data suggest the crucial role of meaning making process in fostering psychological adjustment to cancer illness and treatment.

**1086** POSTER

**Identification of the Receptor Tyrosine Kinase AXL as a New Target for Prostate Cancer Therapy**

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Axl is a receptor tyrosine kinase of the family of TAM receptors (which includes TYRO3 and MER) and plays roles in different types of cancer. It is highly expressed in sarcoma, metastatic colon carcinoma, gastric and certain types of breast cancer. As Axl is upregulated in several metastatic cell types it may play a role during invasion and metastasis. Tyrosine kinases (TKs) represent a major class of proto-oncogenes and are involved in tumour growth, progression and metastasis of cancer cells. TKs are being actively studied as targets for therapeutic intervention and several of them have shown efficacy in clinical trials. Prostate cancer (PCa) is the most common solid cancer in older men and is one of the most frequent causes of deaths. Although androgen ablation therapy, surgery and radiation therapy are effective for the treatment of local PCa, there is no effective treatment available for patients with the metastatic androgen-independent disease. In this work we demonstrated the role of Axl in PCa progression and identified Axl as a potential target for PCa therapy. Using real time PCR to assess the level of tyrosine kinase receptors' expression in PCa cell lines and human tissue, we observed that Axl has consistent over-expression across cell lines and human prostate tumour tissue, providing a model for testing the targeting of Axl. Our data shows a significant increase in Axl expression in metastatic PCa cells and clinical samples (48% of adenocarcinomas of prostate compared with normal prostate tissue). Blockage of Axl gene expression using lentivirus encoding siRNA against Axl inhibits proliferation, migration and invasion of PCa cells. Our pilot studies in a xenograft subcutaneous model demonstrate that inhibition of Axl reduces tumour formation by 50%. Moreover, microarray analysis in addition to pathway analysis of Axl knockdown cells show that some survival pathways are inhibited, but strikingly all members of the NF-kB pathway are down regulated. To establish an alternative for PCa treatment we tested different inhibitors of the NF-kB pathway. Treatment of PCa cells with these drugs reduce proliferation and induce apoptosis. Furthermore, treatment of Axl knockdown cell lines with these drugs enhances their effects. Finally, in order to develop a specific inhibitor for Axl, we are evaluating a library of natural compounds from the African and Asian continents. We have found a compound that reduces proliferation and induces apoptosis in PCa cell lines and reduces Axl levels, thus representing a good candidate for future tests. Taken together our data demonstrates that Axl plays a role in migration, invasion and tumour development and can be used as a marker for invasive and metastatic tumours highlighting it as a target for drug therapies.

**1087** POSTER

**The Synergic Effect of CKD-516 to Conventional Chemotherapy**

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**Background:** Tubulin polymerization inhibitors had emerged as one of promising anticancer therapeutics because of their dual mechanism of action, i.e. apoptosis by cell-cycle arrest and VDA, vascular disrupting agent. VDAs are believed to be more efficient, less toxic, and several of them are currently undergoing clinical trials.
CKD-516 is a vascular disrupting agent (VDA) that attacks only tumour vessels inhibiting microtubule assembly. CKD Pharmaceuticals is conducting a phase I study of CKD-516 in patients having refractory solid cancers in Korea.
The clinical success of VDA inhibitors depends on their combination with other conventional therapeutic agents. In search for new therapeutic modalities to target NSCLC, we investigated the effect of CKD-516 as a single treatment or in combination with the established therapeutic agents such as carboplatin and paclitaxel.

**Materials and Methods:** Xenograft model Tumours were implanted subcutaneously to Balb/c nu/nu mice. After reaching to 100–200 $mm^3$ of tumour volume, nude mice were dosed i.p. with CKD-516 and/or conventional drug on a q.w. schedule. Tumour volume was determined by measuring the diameters of tumours using caliper and calculated using the formula $ab^2/2$, where a is the long length and b is the short length of the tumour. Statistical analysis was performed using the Student's *t*-test.
Toxicity studies CKD-516 and/or conventional drugs are administered to Balb/c mice. Blood samples were collected in EDTA containing tubes for WBC differential counting;, BUN, GPT, GOT analysis.
**Results:** Carboplatin, a second-generation platinum-containing anticancer drug, is currently being used against human cancers but high-dose carboplatin chemotherapy can cause nephrotoxicity in cancer patients.
Low dose CKD-516 inhibited tumour growth significantly in NSCLC xenograft (3 mg/kg, IR = 67.8%). Whereas carboplatin failed to show strong anticancer effect (100 mg/kg, IR = 46.7%) with observable toxicity.
CKD-516 in combination with carboplatin showed a synergistic effect without increasing toxicities (ie, hematologic toxicity, nephrotoxicity, hepatotoxicity).
Paclitaxel is a mitotic inhibitor used to treat patients with lung, breast, ovarian cancer.
CKD-516 inhibited tumour growth against NSCLC xenograft (2.5 mg/kg, IR = 48.7%; 5 mg/kg, IR = 70.4%) without decrease of body weight gain. Whereas paclitaxel inhibited the tumour growth insignificantly (10 mg/kg, IR = 25.2%; 20 mg/kg, IR = 45.5%) with observed toxicity.
CKD-516 in combination with paclitaxel showed synergistic effect without increment of toxicities. The inhibition rate of the combination reached 89.4%.
**Conclusions:** In summary, CKD-516 has shown synergistic effects in combination with other anticancer agents. Thus, CKD-516 warrants further development to treat NSCLC patients.

**1088** POSTER

**Liposome-Encapsulated Hemoglobin Enhances Radiotherapy and Chemotherapy to Suppress Tumour Growth and Metastasis in Mice**

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**Background:** Liposome-encapsulated hemoglobin (LEH) has been developed using technologies for encapsulation of concentrated human hemoglobin with high encapsulation efficiency as well as surface modification to achieve stability in circulating blood and a long shelf life. An affinity of LEH to oxygen can be widely modified chemically. A high $O_2$-affinity LEH ($P_{50}O_2$ = 10 mmHg, h-LEH) showed the improvement in cerebral infarction and cardiac infarction of animal models. As in radiotherapy, anticancer drugs, such as doxorubicin (DXR) but not 5-FU, require $O_2$ to be cytotoxic. We hypothesize that targeted $O_2$ delivery to tumour hypoxia by h-LEH may certainly enhance cancer therapy. The study was performed to assess the potential of h-LEH to overcome tumour hypoxia and to improve the effect of radiotherapy and chemotherapy.
**Material and Methods:** 20 Gy was given to mouse squamous cell carcinoma, SCCVII, grown in the leg of C3H/HeN mice. H-LEH or empty liposome was infused intravenously 30, 60, 90 and 120 min before irradiation. Tumour size was monitored thereafter to evaluate the suppression on tumour growth. H-LEH or empty liposome was infused in the additional mice with SCCVII tumour, which was excised various timing later for immunohistochemical staining for h-LEH and HIF-1α. DXR and S-1 (a novel oral 5-FU derivative) were applied on the Lewis Lung Carcinoma (LLC) grown in the leg of C57BL/6N mice. Daily administration of DXR (0.5 mg/kg, intraperitoneally) or S-1 (8 mg/kg, orally) was started 48 h after inoculation of LLC tumour for 2 consecutive weeks. H-LEH (5 mL/kg) was infused 2 h after each chemotherapy every other day for 2 weeks. After 2W treatments, mice were sacrificed for quantitative and macroscopical examinations of the tumour growth and lung metastasis.
**Results:** H-LEH was most effective when 10 mL/kg was infused before irradiation as compared to empty liposome or 5 or 20 mL/kg of h-LEH. SCCVII tumour growth was most suppressed when interval between h-LEH infusion and radiation was shortest, 30 min. As the result, 10 mL/kg of h-LEH infusion 30 min prior to radiation prolonged 5-time tumour-growth time from 20.0 days (radiation and empty liposome) to 26.5 days; $P < 0.01$, synergy ratio 1.42. H-LEH was detected in the tumour 6 to 24 h after infusion, when HIF-1α expression was reduced only in the h-LEH-treated mice. Administration of h-LEH or DXR alone had no effect on LLC tumour growth in the leg and metastasis in the lung. Addition of h-LEH to DXR resulted in 30.5% reduction of tumour weight ($P < 0.05$) and 41% reduction of lung metastasis ($P < 0.01$). While S-1 had a marked effect on both tumour growth (35% tumour weight reduction) and 62% reduction of metastasis, addition of h-LEH had no synergistic effect on the anti-tumour effect of S-1.
**Conclusions:** These results suggested that h-LEH may have the potential of synergistic action not only with radiotherapy but also with chemotherapy. Decreased expression of HIF-1α in the h-LEH-treated tumour may suggest targeted tumour oxygenation as a potential mechanism.

**1089** POSTER

**Apoptosis Induction by Low Voltage Electric Pulses**

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**Background:** Electric fields have been widely used in a variety of *in vitro* and *in vivo* biotechnical applications. Electroporation, in particular, has been investigated as a novel anticancer therapy, known as electrochemotherapy. However, high-intensity electric fields may also cause undesirable side effects. On the other hand, previous studies reported that a moderate electric field induced apoptosis. Therefore, research into the cellular effects of exposure to mild electric fields is useful for cancer research.
**Materials and Methods:** *Cell culture* The B16 (mouse malignant melanoma) cell line/SCC-9 (human tongue cancer) cell line.
*Exposure to LVEPs (Low voltage electric pulses)* Cells were re-suspended in RPMI-1640 at a concentration of $2-3\times10^6$ cells/ml. The cell suspensions (400 μl) were transferred to electroporation/fusion chambers. Relatively low-voltage (7.5 V/mm) square wave consecutive pulses were applied using a function generator.
*Assay* A flow cytometry apoptosis detection kit (Annexin V-FITC/7-AAD) was used to identify apoptotic and necrotic cells. Caspase-3, -8 and -9 activities were measured using an activity detection kit with FITC fluorescence by flow cytometry.
**Results:** *Induction of apoptosis and cell death*: On average, apoptosis occurred in 2.8% of the control cells, 3.2 −10.5% of cells exposed to electric pulses, and 7.4% of the cisplatin-treated cells.
*Caspase-3, -8 and -9 activity*: Caspase-3 activation increased within 3 h after electric pulse exposure and increased gradually until 24 h. Thereafter, caspase-3 activation decreased gradually, but did not recover to the control level at 48 h. Moreover, LVEPs activate both caspase-8 and -9 (*i.e.,* both the cell death receptor and mitochondrial pathways).
**Conclusion:** LVEPs induce apoptosis in a manner that is primarily dependent upon caspase-3 activation through caspase-8 and -9 activation. LVEP-induced apoptosis may result from membrane dysfunction that disrupts the transport of $Ca^{2+}$ and extracellular substances, which are potent caspase activators. However, further studies are required to define the electric pulse conditions that most effectively induce apoptosis and to elucidate the detailed mechanism of LVEP-induced apoptosis.

**1090** POSTER

**The Polymorphism of the IGF-1 Gene in Patients With Breast Cancer and Effects on Prognostic Factors**

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**Background:** The insulin-like growth factor I (IGF-I) is known to have a role in the risk of breast cancer because of stimulating cell proliferation, differentiation and apoptosis. However, the relation between IGF-I gene polymorphism and the clinicopathological variables of breast cancer remains unknown. We aimed to evaluate the association between CA repeat polymorphism of IGF-I gene in 76 breast cancer patients.
**Methods:** The IGF-I (CA) repeats studied with polymerase chain reaction by using proper primers belonging to these gene areas from DNA samples.
**Results:** Of the 76 patients, 40 (52.6%) had non 19-non 19 homozygote, 12 (15.8%) had non 19−19 heterozygote, and 24 (31.6%) had 19−19 homozygote. There was no relationship between age, body mass index, menopausal status, stage, bilaterality, estrogen receptor status, c-erb B2 overexpression, histological grade, tumour size and the CA repeat polymorphism of IGF-I gene. The non 19- non 19 homozygote were more common in patients without lymph node involvement (70.8%, $p = 0.07$) and in patients without progression (63.2%, $p = 0.044$). In survival analysis, the carriers of non 19- non 19 homozygote had longer PFS (mean 114.3+19 months, $p = 0.13$) and OS (142.9+22.7 months, $p = 0.05$) than the other groups.
**Conclusions:** These results suggest that the non 19-non 19 carriers have some favorable effects on disease progression and survival.