Materials and methods: The hospital records of all the pediatric patients with solid tumors and those who have received palliative radiotherapy from January 1993 to December 2000 were retrospectively analyzed. There were total of 57 patients aged between 1 to 15 years (median 5year) with male to female ratio of approximately 1.9:1. Retinoblastoma was the most common malignancy constituting about 40%; ERMS of head and neck region was next common tumor of about 23%. Other tumors, which diagnosed were neuroblastoma (5 patients), Ewing's sarcoma-3, Hodgkin's disease, NHL and neurofibrosarcoma-2 each, osteosarcoma, hepatoblastoma, esthesioneuroblastoma and malignant melanoma 1 each. The common indications for palliative radiotherapy are locally advanced disease presenting with bleeding, fungation, and ulceration and in some intracranial extension. This group constituted 65% of the cases. Five patients required radiotherapy for cord compression while another 12 patients received RT for bone metastasis, pathological fracture in long bones and brain metastasis in 1 patient.

Of the above patients, 65% have not received any form of treatment before palliative radiotherapy while 35% received some form of treatment, most commonly chemotherapy. The dose of palliative radiotherapy delivered varies from 4Gy, 5Gy and 8Gy in single fraction to 12Gy/3#, 15Gy/5#/1week and 20Gy/5#/1week. 28% of the patients received 20Gy/5#/1week for locally advanced tumor while 8Gy in single fraction was given for bone metastasis and pathological fracture. 4 and 5Gy single fraction treatment was given to control bleeding from primary tumor. While fractionated 12Gy and 15Gy radiotherapy given for spinal cord compression and locally advanced disease. Some patients who showed good response to palliative radiotherapy were further treated with other modality.

Results: Statistical analysis was done using SPSS version 10 for windows soft ware. At the end there were 50 evaluable patients for analysis while 7 patients did not return after palliative RT. The follow up ranged from 4 weeks to 285 weeks (calculated from the time of palliative radiotherapy to last visit). The median follow up was 20weeks. 8 patients (16%) had follow up of more than 1 year. Of these 50 patients 83% had a partial response (both objective and symptomatic) and 17% had not responded to RT (osteosarcoma, neurofibrosarcoma thigh and 2 patient with cord compression). 50% of patients who achieved partial response received further treatment mostly with combination chemotherapy (vincristin, carboplatin, adriamycin, prednisone, etoposide etc). The disease status at the last visit revealed 2 patients died of disease, 5 patients remained disease free (2patients with HD, 1 each of retinoblastoma, ERMS, and hepatoblastoma all with follow up 1 to 5 years and all received further treatment). Rests were alive with symptomatically controlled disease when last seen. Survival analysis by Kaplan-Meier method was done and the survival at year was 22%. There were no major radiotherapy related toxicities. On multivariate analysis there was no significant difference for type of tumor, RT dose and treatment response.

Conclusions: In pediatric solid tumors where the disease is advanced, palliative radiotherapy has a documented role and should be judiciously used for symptom control. Although no single dose schedule is better than other, the dose of RT should be decided taking into account the indications for RT and age of the patient. The above dose schedule showed useful response in >80% of patients and helped about 10% to remain disease free.

955 POSTER

Cancer patients: patterns of internet use

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Background: The Internet becomes increasingly indispensable as a source of information for clinical questions, research, education and patients' interests.

Aim: To evaluate the Internet as a source of medical information for patients with colorectal cancer. The present survey examines the use and the influence of the Internet and other mass media on tumor patients.

Methods: From 07.02.2001 to 23.11.2001, 272 patients with prostate cancer which were referred to the Dept. of Radiotherapy were analyzed using a 36-item questionnaire developed in Freiburg.

Results: Mean age of all patients (n=272): 69 years (range: 35 - 83 years). Level of education (n=247): secondary school 57%(n=142), A-level or college / university: 42%(n=103), other or missing: 1%(n=2). Occupation (n=258): pensioner: 78%(n=201), employees: 10,5%(n=28), worker: 4%(n=10), self-employee: 5%(n=13), houseman: 0,5%(n=1), other or missing: 2%(n=5). Access to computer (n=255): yes: 16%(n=42), no: 69%(n=176), access to Internet: 15%(n=37). Frequency of Internet use (n=30): weekly: 0%(n=0), monthly: 13%(n=4), occasionally: 37%(n=11), rare: 20%(n=6), never:30%(n=9). Reasons of not having/using a computer or the Internet (n=161): fear of high tech: 17%(n=27), too time-consuming: 5%(n=9), too expensive: 25%(n=40), other reasons: 53%(n=85). Making use of other information sources than the doctor treating the patient (n=230): 46%(n=106). If the layman-system was used as an information source they used as an information source (n=96): Internet: 20%(n=20), other prostate cancer patients: 31%(n=31). Reliability of informations: ARD+ZDF(n=215): high: 54%(n=108), Internet(n=118): high: 22%(n=26), taxidriver(n=152): high: 11%(n=17).

Conclusions: The importance of the medium Internet as a source of information for tumor patients with prostate cancer in Freiburg is currently still low but likely to increase. The percentage of internetuser in the "normal population" over 50 years is only about 16%. Only 5% of our patients have visited the homepage of the department of radiotherapy at the University Clinic of Freiburg (http://www.ukl.uni-freiburg.de/rad/strahlen/homede.html or short cut: http://go.to/radiotherapy). The demographic structure and a further spread of Internet-access will lead to a gain of popularity of the Internet among prostate cancer patients.

The project is presented online: http://www.krebsmedizin.info/index.php.

956 POSTER

Websolution for the prescription of antineoplastic drugs.

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Background: The Department of Oncology carried out a systematically survey concerning errors and near errors in the administration of drugs from September 2001 until June 2002.

14 errors and 16 near errors were reported. A total of 18 errors came from the administration of antineoplastic drugs.

The errors were divided in the following categories: • Incorrect drug • Incorrect dose • Incorrect timing · Incorrect preparation of the drugs • Other

Aims: The aim was to develop a web based solution which could minimize errors related to drug delivery and at the same time fulfil the following issues: • Easy to read requisitions • Easy to add and to update patient information • Automatic calculation of drug doses, drug reduction and escalation

Technical Solution The solution uses MS IIS, MS SMTP, MS Transaction server, MS SQL server and an ActiveX component developed by us. The generated documents are in Active Sever Pages and PDF formats.

Software Solution: The web solution is built on a MS SQL database, which contains information about users, treatments, standard doses and preparation of the drugs and patients data. The nurses are responsible for adding patient's data to the system based on the doctor's instructions and to update the patient data.

Prescriptions are automatically printed to the Pharmacy laboratory. As an extra precaution the prescriptions are also emailed as attached PDF documents. Through a number of pre-designed reports, the system will allow all the involved personnel to continuously monitor the drug prescriptions.

Conclusion: Changing from a manual system to electronic requisition has resulted in: • Development of precise standard operational procedures (SOP) • Easy access to all requisitions • Minimizing the number of errors and near errors. • Made it possibility to monitor the drug accumulation

Especially the Pharmacy has been satisfied by the ease in which they have access to all the requested antineoplastic. now in and readable and correct form. In the clinic there has been a clear indication that patients prior to the implementation of the system were given incorrectly doses of drugs, these errors have now been resolved by the system.

Molecular targeted therapy

957 POSTER

Ectopic expression of the amino-terminal peptide of androgen receptor leads to androgen receptor dysfunction and inhibition of androgen receptor-mediated prostate cancer growth.

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Background: Androgen receptor (AR) is a ligand-activated transcription factor that requires androgen binding to initiate a series of molecular events leading to specific gene activation. AR has been suggested to

form an antiparallel homodimer based on the characteristics of high affinity interaction between the amino (N) and carboxyl (C) termini of the receptor. Recently, it is suggested that AR N-to-C interaction is critical for the ability of this receptor to up-regulate the transcription of androgen-responsive genes, and may be a new target for treatment of prostate cancer. In this study, we investigated the effect of N-terminal (1-34) peptide of AR (ARN34) on androgen-dependent function in prostate cancer cell.

Material and methods: We constructed a plasmid, pTriARN34, expressing ARN34 by cytomegalovirus promoter. To measure the *in vivo* interaction of the amino terminal domain and ligand-binding domain of AR, we used the mammalian two-hybrid system. Stable clones of LNCaP cells expressing ARN34 were selected with medium containing of G418.

Results: Transfection of pTriARN34 suppressed dihydrotestosterone (DHT)-dependent N-to-C interaction of AR in a dose-dependent manner. On AR-mediated reporter gene assay, the expression of ARN34 suppressed DHT-dependent prostate specific antigen transcription. ARN34 also suppressed AR nuclear translocation induced by DHT. Stable expression of ARN34 suppressed androgen-dependent cell growth of LNCaP cells. Moreover, this inhibitory effect of ARN was also confirmed in hydroxyflutamide-induced mutated AR transactivation and cell growth. Treatment of LNCaP cells with 1 nM DHT drove transition of cells from G1 to S-phase. On the other hand, the ectopic expression ARN34 led to cell cycle arrest by inhibiting the entry into S phase in LNCaP cells.

Conclusions: Our results demonstrate that disruption of AR N-to-C interaction caused by ARN34 leads to AR dysfunction and inhibition of AR-mediated prostate cancer cell growth. This approach is thus considered to provide a useful therapeutic opinion for blocking AR-mediated prostate cancer growth.

958 POSTER

Adenoviral transfer of a natural antisense to survivin mRNA down-regulates survivin expression and promotes apoptosis in breast cancer

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Background: Survivin, a member of the inhibitor of apoptosis (IAP) family of proteins, is mostly expressed in malignant cells in adult and recognized as a good target for cancer gene therapy. Previously we demonstrated that induction of a natural antisense of survivin, effector cell protease receptor-1 (EPR-1) down-regulated survivin expression with decrease of cell proliferation, increase of apoptosis, and increase of sensitivity to anticancer agent (Yamamoto et al. European Journal of Cancer, 2002; 38:2316). In this study, we constructed a replication defective adenoviral vector encoding the same antisense sequence to survivin and attempted to enhance the efficacy of previous study. By demonstrating an effect of survivin modulation, we ultimately would like to explore a strategy of gene therapy only toxic to malignant cells expressing survivin.

Material and methods: Breast cancer BSMZ cell line was established by one of the authors (Watanabe et al. Cancer Research, 1992; 52:5178). An adenoviral vector encoding antisense RNA to survivin was constructed by homologous recombination of adenovirus type 5-derived pJM17 and shuttle plasmid, pCMV-EPR-1 in HEK 293 cells.

Results: We infected the vector to BSMZ cells with multiplicity of infection (MOI) of 0, 1, or 5. Cells were harvested, then transcription and expression of survivin were monitored. Northern blot demonstrated that signals of transduced EPR-1 increased MOI-dependently. Correspondingly, cellular levels of survivin decreased 72-hours after viral infection. In cell cycle analysis, down-regulation of survivin caused increased population in the fraction of apoptotic cells (sub-G1 peak) (MOI=0: 4.88%, MOI=1: 5.05%, MOI=5: 11.54%) with decrease in the S phase population (8.78, 9.94, 10.28%, respectively). Cytotoxic assay revealed that transduction of antisense conferred MOI-dependent sensitivity of docetaxel, a chemotherapeutic agent to BSMZ cells.

Conclusions: Current study demonstrated that adenoviral transduction of antisense sequence to survivin mRNA down-regulated survivin expression and increased apoptotic fraction. Moreover, it sensitized cells to docetaxel. Since survivin is expressed primarily in malignant cells, our results suggest possible cancer gene therapy with no adverse effect on normal tissues which do not express survivin. Enhancement of chemosensitivity by modulation of survivin may also have a roll for further development of therapies to drug-refractory malignant tumors.

959 POSTER

Expression of functional CXCR4 on colorectal human cancer.

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Background: The chemokines are small proteins known to direct hematopoietic cells to home-specific anatomical sites. The chemokine receptor for SDF1-alfa chemokine, CXCR4, has been implicated in cancer metastasis. Emerging data suggest that it has a key role in determining the metastatic destination of tumor cells as demonstrated in breast, melanoma, ovary, and lung cancer. Since the expression of the CXCR4 receptor may be of prognostic value we studied the expression of CXCR4 on human colorectal cancer.

Methods: CXCR4 expression was examined by immunohistochemical staining on paraffin-embedded sections of normal colorectal mucosa (14), iperplastic polypi (6), dysplastic polypi (27), 16 primary carcinomas and 5 hepatic metastasis. CXCR4 expression was also studied by flow cytometry on Caco2, GEO, SW480, SW48, Lovo and SW620 human colorectal cancer cell lines. The effect of SDF1-alfa and liver-derived proteins on migration of cell lines was measured using transwell inserts (8 1/4 m diameter) and 24-well plates. The inhibitory effect of anti-CXCR4 antibody (10 1/4 g/ml) on migration was also studied.

Results: CXCR4 staining resulted weakly positive in 6 and strongly positive in 1 (infiltrated by melanoma) out of 14 samples of normal mucosa, clearly positive in 19 out 27 dysplastic lesions with higher staining intensity for moderate/poorly differentiated lesions (13/17, moderate/poorly vs 6/10, well differentiated), and dramatically positive in 16 out of 16 carcinomas.

SW480, SW48 and SW620 human colon cancer cell lines showed the highest levels of the CXCR4 (60-80% of positive cells), 30-60% for Caco2 cells, 20% Lovo cells and 5-10% GEO cells compared to the 50% of the MDA231 human breast cancer cell line considered to be an ephitelial cell line overexpressing CXCR4 and to the 8% of the HT1080 human fibrosarcoma cell line.

In order to verify the functional status of CXCR4, the ability to migrate versus its natural ligand was assayed on SW480 human colon cancer cells. Preliminary results showed that SW480 migrate in response to SDF1-alfa chemokine relatively to the expression of CXCR4. Furthermore the neutralization of CXCR4 by antibodies inhibits *in vitro* the migratory response to purified SDF1-alfa as well as to liver-derived proteins. Thus the overexpressed CXCR4 is functional.

Conclusions: These preliminary results showed CXCR4 overexpression on human colon cancer tissue compared to normal mucosa and benign lesions. Experiments on human colon cancer cell lines suggest a functional activity of CXCR4. Studies on the possible prognostic role of the CXCR4 expression in patients bearing colorectal cancer and its eventual role for targeted therapy are warranted.

960 POSTER

Therapy of MHC class I+ and class I- HPV16-associated tumours with IL-2, IL-12, and genetically modified tumour vaccines

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Purpose of the study: To examine local and systemic effects of IL-2, IL-12 and genetically modified tumour cell-based vaccines directed against HPV16-associated neoplasms in experimental model systems.

Experimental models: Moderately immunogenic, MHC class I-negative MK16/1/IIIABC (MK16) cells were previously established by co-transfection of HPV16 E6/E7 and activated Ha-ras DNA into C57 BL/6 murine kidney cells. The MK16 cells formed s.c. tumours in syngeneic mice and metastasized to lungs and lymph nodes (Smahel, Sobotkova, Bubenik et al., Br. J. Cancer 84:374-380, 2001). For comparison, MHC class I-positive, non-metastasizing TC-1 cells, established by co-transfection of C57BL/6 murine lung cells with E6/E7 HPV16 and activated Ha-ras DNA (Lin, Guarnieri, Staveley-O'Carroll et al., Cancer Res. 56:21-26, 1996) were utilized.