Complete response rates at 1, 3, 6, 12 months were 43%, 73%, 76% and 93% for 8 Gy and 32%, 62%, 60% and 55% for 6 Gy. There was significant difference between complete response rates of 8 Gy or 6 Gy at 12 months ($p < 0.001$). Side effects were tolerable in all but 3 patients who experienced fibrosis and edema.
Conclusion: Radiotherapy is an effective mode of treatment for localized Kaposi's sarcoma and it seems that single dose of 8 Gy is more effective than 6 Gy for long-term local control.

1219 PUBLICATION

Methionine-free diet in association with nitrosoureas treatment of metastatic melanomas: Methionine-free diet duration and modulation of O6-methylguanine-DNA-methyltransferase

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Background: In *in vitro* and *in vivo* experiments, Methionine (MET) depletion used in association with cytostatic drugs has been shown to improve of their therapeutic index. One hypothesis by which MET deprivation sensitized these tumors to nitrosoureas (CENUs), could be attributed to down-regulation of the repair protein O6-methylguanine DNA methyltransferase (MGMT), one of the main mechanisms of resistance to CENUs. On the basis, we initiated a phase I clinical trial associating dietary MET restriction with nitrosourea treatment (cystemustine) for metastatic melanomas. We determined the optimal MET-free diet duration and evaluated the feasibility of this association and its impact on MGMT activity in peripheral blood mononuclear cells (PBMCs) during treatment.
Material and methods: Ten patients received 2 months of treatment, i.e. 4 cycles every two weeks of the association of MET-free diet and nitrosourea treatment (Cystemustine 60 mg/m^2). During each cycle, patients received standard diet the 1rst day and then MET-free diet, which allowed testing randomly 4 periods of 1, 2, 3 or 4 regime days. Daily concentrations of plasma MET, before and after diet nutritional status (BMI and PINI determinations) and toxicity were evaluated. Every cycle, MGMT activity level was measured by HPLC in PBMCs isolated on ficoll from blood samplings before and after diet period.
Results: Dietary MET restriction reduced MET concentrations from 21.21.3 μM before diet to 12.01.0 μM from only one day of diet, with a mean optimal decline of 41%. No cumulative effect have been observed despite the lengthening of MET-free diet duration. MET-free diet have not deleterious effect on nutritional status. The toxicity OMS grade 3–4 remained moderated (3/10 thrombopenia and 3/10 neutropenia). Comparing before and after diet period (analysis of 6 patients), the MGMTactivity in PBMC of these patients was not affected by MET restriction (434±108 fmol/mg before MET-free diet vs 354±49 fmol/mg after MET-free diet). Individual interpatient variability of MGMT activity was very important, ranged from 83 to 1424 fmol per mg of protein. However, plasma MET variation (before and after diet) seemed to be correlated with the MGMT activity variation.
Conclusions: A 1 day MET-free diet will be adopted to realise a phase II clinical trial aimed at evaluating the therapeutic efficacy and toxicity of the association of MET restriction diet with nitrosourea treatment. Concerning modulation of MGMT activity, these preliminary results might be confirmed and would be explored on phase II clinical trial patients.

1220 PUBLICATION

Intraoperative electron beam therapy (IOERT) combined with EBRT in the treatment of retroperitoneal sarcomas

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Purpose: Complete surgical resection remains the mainstay of treatment of soft tissue sarcomas (STS) located in the retroperitoneal space, but is possible only in 60–70% even in patients presenting with primary disease. Randomized trials have demonstrated improved local tumor control for patients with extremity STS after postoperative radiotherapy (EBRT) whenever doses of 60–70 Gy can be administered. As EBRT in retroperitoneal STS is limited by tolerance doses of surrounding tissues, additional IOERT was used to overcome these dose limitations.
Methods: From 1991 to 2003, 67 patients (m/f 38:29) with retroperitoneal STS (primary disease 31, recurrent disease 36) were treated with maximal resection, IOERT and EBRT. Median age was 54 years. Tumor grading was G1 in 5 (7%), G2 in 20 (30%) and G3 in 42 patients (63%). Mean tumor size was 10.5 cm. Most common histology was liposarcoma (50%). Mean IOERT dose was 15 Gy. Electron energies ranged from 8 to 15 MeV. IOERT was delivered to the complete tumor bed if possible. Postoperative EBRT was given to 94% of our patients with doses of 39.6–50.4 Gy (mean 42.5 Gy) in 1.8 Gy per fraction. EBRT was delivered by linear accelerator with photon energies ranging from 6 to 23 MV after CT-based 3D treatment planning.
Results: Median follow up was 20 months. Complete resection was possible in 21 (31%) patients, while 34 patients (51%) showed microscopically, and 12 (18%) patients macroscopically residual disease. 5 year actuarial overall survival was 52%. Primary vs. recurrent status had no significant impact on survival. Actuarial 5 year survival was marginally significantly affected by tumor grade (80% for G1/2 vs. 41% for G3 tumors, $p = 0.06$). After complete resection, 5 year actuarial survival was 70% compared to 45% after R1- and 34% after R2-resection. Actuarial 5 year metastatic-free survival was 54%. The 5 year actuarial local control rates in- and outside the IOERT field were 78% and 54%. Except for 5 patients with postoperative wound healing disturbances, no IOERT related acute complications were seen. Only 2 patients required surgical interventions due to late complications.
Conclusion: Combination of maximal surgical resection, IOERT and EBRT in patients with retroperitoneal sarcomas results in good overall survival, especially in R0 resected patients, without increased toxicity to normal tissue.

1221 PUBLICATION

Genetic characterisation of leiomyosarcoma

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Leiomyosarcoma (LMS) is a malignant tumour of mesenchymal origin displaying phenotypic features of smooth muscle differentiation and accounting for approximately 5–10% of soft tissue sarcomas. Soft tissue sarcomas (STS) are relatively rare compared to epithelial cancer comprising less than 1% of all human cancers.
LMS falls into a group of STS which frequently show complex karyotypic changes and extensive heterogeneity characteristic of severe disturbances in genomic stability. With an overall low long term survival rate, a lack of a tumour specific genetic alteration, and a wide spectrum of histopathological features and clinical behaviour, progress in the diagnosis, classification and management of these tumours has been limited.
Comparative genomic hybridisation (CGH) was used to characterise a series of paraffin embedded cases of LMS to identify candidate regions containing tumour relevant genes involved in the development of LMS. CGH provides information on regions of amplification and deletion of genetic material across the whole tumour genome in a single hybridisation experiment.
The results have shown chromosomal gains to be more frequent than losses. Among the most common gains and losses, a high frequency of gains were present on 1q, 4q and 6q, and a high frequency of losses involved regions on chromosomes 1p, 8p and 19q. New regions of chromosomal gain and loss occurring in high frequency have also been highlighted. The results suggest these regions of gain and loss may contain oncogenes and tumour suppressor genes, respectively, involved in the development and progression of LMS, and are subject to further investigation.

1222 PUBLICATION

Development of a rapid screening approach for candidate gene sets in cancer

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Background: During the last decade, microarray-based gene expression analysis gave rise to a large number of candidate genes for the diagnostics and therapy of cancer. Bioinformatic approaches delivered gene sets, the expression patterns of which were predictive for certain cancer phenotypes. A synergy between these advances and the development of screening tools for a rapid and reliable screening of marker gene expression represents an important step towards an improved treatment of cancer.
Methods: For the semi-quantitative expression screening of eleven candidate genes for drug resistance in melanoma, we combined multiplex RT-PCR (mRT-PCR) with subsequent microfluidic fragment analysis.
Results: The functionality of this approach was demonstrated by low inter-experimental variations of amplicon quantities after endpoint analysis. Applied to RNA samples derived from drug-sensitive and -resistant melanoma cell lines, mRT-PCR delivered results qualitatively concordant

with data obtained from Northern blot- and array-analyses. A preliminary screen of four additional melanoma cell lines points to *IL1B*, *APOD*, and *CYR61* as interesting candidates for drug-resistance associated genes. First tests using an automated on-chip electrophoresis platform indicate the applicability of this approach for high throughput measurements.
Conclusion: mRT-PCR combined with on-chip electrophoresis reveals a rapid and easy-to-handle method for candidate gene set evaluation from limited amounts of mRNA. Using gene sets indicative for different tumor phenotypes, this procedure may represent an alternative for future cancer diagnostics.

1223 PUBLICATION

Antisense-mediated downregulation of ML-IAP sensitizes melanoma cells to chemotherapy

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Background: Advanced malignant melanoma is an aggressive form of skin cancer which is highly resistant to standard anticancer agents. ML-IAP(melanoma inhibitor of apoptosis) is a potent inhibitor of apoptosis which is strongly upregulated in melanoma, while being undetectable in most normal tissues including normal melanocytes. Targeted downregulation of ML-IAP thus has potential to sensitize refractory melanoma to chemotherapy.
Materials and methods: We designed 20-mer phosphorothioate antisense oligonucleotides(AS-ODNs) complementary to five single-stranded target sites on the ML-IAP mRNA using a computer-based secondary structure prediction program. G361 and SK-MEL28 melanoma cells were transfected with AS-ODNs in the presence of cationic lipids. Inhibition of ML-IAP mRNA and protein expression were measured by real-time PCR and immunoblotting, respectively. Sensitization of cells to chemotherapy was detected in cell growth assays using the anticancer agent cisplatin.
Results: M706 was identified as the most efficient AS-ODN, which downregulated ML-IAP mRNA by 68% and 54% in G361 and SK-MEL28 cells, respectively. The specificity of target downregulation was confirmed using scrambled and mismatch sequence controls, which only marginally decreased ML-IAP mRNA levels in the cell lines. In addition, compared to transfection with control oligonucleotides, downregulation of ML-IAP using AS-ODN M706 resulted in more than 2-fold increase in cytotoxic effect of cisplatin on melanoma cells.
Conclusion: We describe a new antisense oligonucleotide that effectively downregulates ML-IAP expression and sensitizes drug resistant melanoma cells to chemotherapy. Our data warrant further investigations to define the therapeutic potential of ML-IAP antisense in the treatment of chemoresistant melanoma.

Paediatric Oncology

Oral presentations (Wed, 2 Nov, 9.15–11.15) **Paediatric oncology**

1224 ORAL

Radiotherapy in pediatric atypical rhabdoid/teratoid tumours of the CNS (CNS-ATRT) – results from the German HIT-data base

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Background: Atypical teratoid/rhabdoid tumours of the central nervous system (CNS-ATRT) are an extremely rare and aggressive, embryonal tumour entity of early childhood. Due to their rarity there is yet no standard therapy, prognosis is poor. Published reports mainly focus on chemotherapy regimen (ChX). The role of radiotherapy (RT) has yet not been analysed in detail, recommendations for RT have not been defined so far.
Material and methods: We report on patients with CNS-ATRT enrolled in the German HIT-study data base (GPOH) between 1988–2004. Clinical records were reviewed retrospectively with special regard to RT data and survival times. Statistical analysis was performed for overall survival (OS) and progression free survival (PFS) concerning 1. the role of RT compared to chemotherapy (ChX-pat. vs. RT-pat.) 2. the sequence of RT in clinical course (RT in primary therapy (primRT) vs. RT in relapse therapy (relRT)) 3. the radiation field necessary for local tumour control (involved field RT (focRT) vs. craniospinal RT (CSA-RT)). Distributions were estimated using Kaplan-Meier plots and log-rank test for significance.
Results: 64 pat. were diagnosed during a 16-year-interval. 59/64 (92.2%) have been centrally reviewed for histology. 29/64 pat (45.3%) had ChX solely, 35 pat. (54.7%) received combined RT/ChX. 45/64 pat. (70.3%) were younger than 3 years at Dx with RT/CHX in 18/44 cases (40.9%). In the age group over 3 years at Dx (n = 19) RT was delivered in 17/19 pat. (89.5%). In 18/35 cases (51.4%) RT was part of primary therapy, in 17/35 part of relapse therapy. RT target volume: 12 × focRT, 21 × CSA-RT, 2 no inf.. RT fractionation/total tumour dose (TTD): conventional fract. RT (n = 31): 54.6 Gy (44.5–59.4), CSA-dose 24–35.2 Gy; hyperfractionated RT (n = 2): 68/71 Gy, CSA-dose 36 Gy; radiosurgery (n = 1): 16 Gy. Survival analysis: 2-year-OS of pat. with combined RT/ChX (56.2%) was significantly better than that for pat. receiving ChX solely (9.2%); p = 0.001. There was no significant difference in 2-year-PFS (from date of RT) concerning sequence of RT (primRT-pat. (42.8%)/relRT-pat. (36.2%)); p = 0.4230. No difference was found in median PFS (from date of RT) concerning radiation field (focRT vs. CSA-RT) in local disease.
Conclusions: 1. RT should be part of treatment in CNS-ATRT. 2. RT at relapse is probably equivalent to RT in initial therapy. 3. Focal RT is probably equivalent to CSA-RT concerning tumour control in local disease.

1225 ORAL

Radiochemotherapy of pediatric atypical teratoid/rhabdoid CNS-tumors: an interim analysis of the German ATRT-CNS pilot study

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Background: The atypical teratoid/rhabdoid tumor (AT/RT) is a very rare CNS-tumor of newborns and infants. The overall survival is exceptionally poor (median: 6–11 months). No controlled treatment study has been published. An anthracyclin-based chemotherapy (Ctx) was effective to shrink the tumors but not to cure. In most series radiotherapy (RT) improved the outcome. However, there was no advantage of neuroaxis or whole brain RT compared with local RT. Therefore, based on the German pediatric survival data (28 eligible children) in the years 1990–2004 and on a meta-analysis of the outcome of treated children (64 case reports) in the years 1986–2004, we developed a novel anthracyclin-based multi-modality therapy including a local RT.
Patients and methods: Children are enrolled in this study if the diagnosis of ATRT of the CNS was confirmed by the German Neuropathology Reference Center. After two induction Ctx cycles (doxorubicin 25 mg/m^2/d, 12 h i.v., d 1–3; dactinomycin 45 μg/kg/d, i.v. push, d 1; cisplatin 70 mg/m^2/d, 6 h i.v., d 4; vincristine 1.5 mg/m^2/d, i.v. push, d 8, 15; methotrexate 2.0 mg single dose intrathecal, d 1–4) a high conformal local RT (54 Gy, 5 x 1.8 Gy/w) with simultaneous Ctx (carboplatin: 80 mg/m^2/d, 6 h i.v., d 1–4) was given. Due to the youth of the patients we choose a safety margin of only 0.5–1 cm around the GTV to define the PTV). Thereafter a reinduction Ctx cycle (same as 1st and 2nd cycle) was implemented. Next, a consolidation Ctx (6 cycles/9 months: CCNU 75 mg/m^2/d, d 1; cisplatin 70 mg/m^2/d, d 1; vincristine 1.5 mg/m^2/d, d 1, 8, 15; methotrexate 2.0 mg single dose intrathecal, d 1–4) was started.
Results: In 10 of 14 children (11 m., 3 f.; median age 11 months) data were available. Primary surgery: 1 SR, 7 PR, 2 biopsy. After induction Ctx, in 9 of 10 children (one died) a response was observed (1 CR, 7 PR, 1 SD). Two children completed the study and showed NED since 22 respectively 33 months after diagnosis. One child (12 month at RT) developed (4 month after RT) a radionecrosis within the PTV. However, he had no clinical symptoms and the MRT's showed no progression of the necrosis 21 month after RT.
Conclusion: The treatment results are encouraging. The induction Ctx is effective but toxic. High doses of RT+intrathecal MTX in infants can cause necrosis. However, this concept is firstly justified by the high risk