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

of lethal tumor recurrence. Secondly, we expect rather radiological visible than clinical relevant side effects due to the very small PTV definition of our concept.

1226 ORAL

Prospective cooperative multicenter study SIOP/GPOH "low grade glioma" in childhood (1996): results after Radiotherapy (German cohort)

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Background: The prospective trial HIT SIOP LGG 1996 was the first in Europe offering a uniform concept for observation and treatment of children with low grade glioma (LGG). We evaluated the German cohort of patients treated with Radiotherapy (including patients from Switzerland, Austria and Belgium).
Methods: Stratified by age, children with LGG started non-surgical therapy after tumour resection or clinical diagnosis only when the tumour was progressive or clinically symptomatic: Chemotherapy (Chx) was performed, if age was under 5 years, or Radiotherapy (RT), if children were older. Both Brachytherapy (BT) and percutaneous fractionated RT were permitted. We evaluated data for survival (Kaplan-Meier-Analysis), RT technicque and acute toxicity. 144 Pat. received RT (RT-group) as first non-surgical treatment, 33 children after failure of initial Chx (Chx-RT-group).
Results: In the RT-group 91/144 patients (63%) had percutaneous RT, while 53/144 patients (37%) had BT with Jod-125-seeds. The corresponding numbers in the Chx-RT-group were 32 and 1, resp. The median follow up time from beginning of RT was 35 months in the RT-group and 32 months in the Chx-RT-group (range 0.3–94 and 1–88, resp.). There was no relevant difference in progression free survival (PFS) after 3 years in both groups (RT-group 70%, Chx-RT-group 68%). Radiotherapeutic technique didn't show a relevant influence (3-year-PFS percutaneous RT 68.5%, BT 73.5%, p = 0.29). By defining age groups of children <1 year, 1–4 years, 5–10 years and >10 years at RT, a bad outcome for the very young children (<1 year, 3-y.-PFS 33%), a good outcome for the children aged 1–4 years (3-y.-PFS 89%) and a further decrease with age (5–10 y. 71%, >10y. 62%, p = 0.027) was found. There were only few cases of 3–4-acute toxicity: 2 × dermatitis grade 3, 1 × leukopenia 3, 1 × headache 3, 1 × local catheter infection, 1 × suspected radiation induced encephalitis, 1 tumourradionecrosis, 1 × local infection of the BT katheter and some other temporary problems like dysacusis and visual impairment (together n = 6).
Conclusions: RT for LGG in children is effective and good tolerable, even after failure of Chx. Prospective studies like the subsequent SIOP GPOH LGG RT 2004 trial (active in Germany since 01.04.2004) are necessary in order to evaluate the value of the RT within a comprehensive treatment concept, esp. regarding the role of modern irradiation technicques and the incidence of relevant late toxicities.

1227 ORAL

Treatment results of 165 pediatric patients with non-metastatic nasopharyngeal carcinoma: a rare cancer network study

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Purpose: This Rare Cancer Network study was performed retrospectively in pediatric (age ⩽17 years) NPC patients to evaluate the role of chemotherapy, the optimal dose of radiotherapy, and the differences in outcomes in regard to the possible prognostic factors.
Material and methods: The study included 165 (109 male and 56 female) pediatric patients with the diagnosis of non-metastatic NPC treated between 1978 and 2003 from 16 centers collaborating in the Rare Cancer Network. Only patients evaluated either with CT or/and MRI for loco-regional tumor were included in the study and were staged according to AJCC 1997 classification. The median age is 14 years old (Range 7–17 years). Histopathological classification revealed 23 (13.9%) patients with WHO II and 142 (86.1%) patients with WHO III. Cranial nerve palsy was present at diagnosis in 12 (7.2%) patients. There were 3 (1.8%) patient with stage I, 1 (0.6%) with IIa, 10 (6.1%) with IIb, 60 (36.4%) with III, 44 (26.7%) with IVA, and 47 (29%) with IVB. All patients were treated with fractionated external beam radiotherapy (EBRT) to a median dose of 66 Gy (Range:49.8–74.4 Gy). Chemotherapy schedule was non-cisplatin based mono therapy in 1 (0.8%), cisplatin based mono therapy in 10 (6.9%), non-cisplatin based multi regimen in 29 (20.1%) and cisplatin based multi regimen in 104 (72.2%) patients. The median follow-up time for all patients was 48 months (Range, 5–249 months).
Results: The actuarial overall 5-year survival (OS) was 77.4%, whereas the actuarial 5-year local relapse free survival (LRFS), loco-regional relapse free survival (LRRFS), distant metastasis-free survival (DMFS) and disease-free survival (DFS) rates were 87.8%, 81.9%, 80.5% and 68.8%, respectively. In univariate analysis, statistically significant unfavourable factors were male gender for DMFS (p = 0.01), T3&T4 disease for LRFS (p = 0.01), presence of cranial nerve palsy at diagnosis for LRFS (p = 0.02) and LRRFS (P = 0.01), stage IV for DFS (p = 0.02), N3 disease for DFS (p = 0.004) and OS (p = 0.03), total nasopharyngeal EBRT dose of less than 66 Gy for LRRFS (p = 0.01) and patients treated with radiotherapy alone for LRFS (p = 0.001) and LRRFS (p = 0.02). In multivariate analysis, statistically significant unfavourable factors were age older than 14 years for LRC (p = 0.04, RR:2.3); male gender for DMFS (p = 0.03, RR:2.7); T3, T4 disease for LRFS (p = 0.01, RR:6.2); N3 disease for DFS (p = 0.002, RR:2.4) and OS (p = 0.002, RR:2.4); total nasopharyngeal EBRT dose of less than 66 Gy for LRFS (p = 0.02, RR:3) and LRRFS (p = 0.002, RR:3.8); and patients treated with radiotherapy alone for LRFS (p = 0.0001, RR:5.9), LRRFS (p = 0.007, RR:3.4) and DFS (p = 0.02, RR:2.2).
Conclusion: We have defined favourable prognostic factors in our pediatric NPC cohort as younger age (age < 14), female gender, early T1, T2 and N0–2 status, total nasopharyngeal EBRT dose ⩾66 Gy, and the treatment schedule incorporating chemotherapy with radiotherapy. This data suggests that high dose RT combined with multi-agent chemotherapy is effective in achieving satisfactory results.

1228 ORAL

Results of Spanish Cooperative Protocol SEOP-95 for non metastatic osteosarcoma of the limbs in children

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Background: To improve the survival and the rate of limb salvage procedures the Spanish Society of Pediatric Oncology (SEOP) started in 1995 the SEOP-95 protocol. From 1995 to 2000, 100 patients with non metastatic osteosarcoma of the limbs were enroled.
Material and methods: The protocol consisted of preoperative chemotherapy with Ifosfamide, Doxorubicin, Cisplatin and high dose Methotrexate for 14 weeks. After surgery, patients received chemotherapy with the same drugs for 25 weeks.
Results: Median age was 12 years (range 4y-20y) and there were 54 males and 46 females. Femur (50%), tibia (32%) and humerus (11%) were most common primary sites. Compliance to pre and post-chemotherapy was >90%. Limb salvage procedures were performed in 85 patients (85%) and mutilant in 15. Good histologic response (>90% necrosis) was observed in 67 cases. There were 7 local relapses (7%) and 4 treatment-related deaths (1 venoclusive disease and 3 sepsis). One patient developed a second neoplasia (AML). Three patients suffered moderate hipoacusia. No renal or cardiac sequelae were observed. With a follow up of 48 to 124 months (median 89 months) the actuarial 5 year event-free-survival is 67%. Survival for good histologic response patients was 73% versus 52% for poor responders (P = 0.011). Survival for patients with tumor volume <100ml was 80% versus 68% for the rest (P = 0.12).
Conclusions: This protocol led to good oncologic and orthopedic results, with a significant treatment –related mortality. Our current protocol SEOP-2001 has shortened the post-op chemotherapy, in order to reduce treatment-related toxicity.