near-diploid/tetraploid. In this study we approached the biological basis of ploidy in order to unveil the genetics behind each relevant subgroup of NBT.
Material and Methods: 49 NBTs with available ploidy data were evaluated by microarray gene expression analysis. Genes with high expression variability were selected by determining standard deviations higher than a factor 2- and 3-fold the sample means. Differential gene expression was validated on 46 independent primary NBTs using quantitative real-time PCR (qPCR). Concomitant gene copy number analysis was performed on 27 out of these 46 NBTs by qPCR to ascertain the correlation between gene expression level and DNA copy number.
Results: Pair wise comparison analysis of near-triploid versus near-diploid/tetraploid NBTs revealed 254 statistically differentially expressed genes capable of significantly discriminate between the 2 groups. A large set of these genes mapped to chromosomal regions with described recurrent abnormalities in NBTs; chromosome 1, 36/254 genes ($p = 0.01$); chromosome 17, 33/254 genes ($p < 0.0001$); and chromosome 19, 26/254 genes ($p = 0.05$).
To validate microarray gene expression data, 13 genes on chromosomes 1 and 17 were analyzed in 46 NBTs by qPCR. The expression levels identified by qPCR confirmed the microarray data; 77% of the analyzed genes maintained statistically significant differences between groups.
Four genes on chromosomes 1 and 17 were further analyzed for gene copy number by DNA qPCR. Gene copy number results in near-triploid NBTs were suggestive of tri- or tetrasomies, while near-diploid/tetraploid NBTs displayed normal somies.
Comparison between DNA copy number and gene expression levels revealed a higher expression in near-triploid NBTs in contrast to a lower expression in near-diploid/tetraploid NBTs than expected according to gene copy number.
Conclusions: Gene expression profile analysis of NBTs with different DNA content revealed a relevant list of genes differently expressed mapping at specific chromosomal regions. No correlation between gene expression levels and DNA copy number was found, suggesting specific tumorigenic transcriptional regulation mechanisms other than gene dosage effect.

1402 ORAL

Body mass index in adult childhood cancer survivors after treatment with potential cardiac and vascular toxicity

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Background: We performed a cross-sectional study on cardiovascular status in childhood cancer survivors (CCS) who received treatment with potential cardiac and vascular toxicity. Since overweight is considered an important risk factor for cardiovascular disease, we evaluated body mass index (BMI; weight/height^2) at several timepoints post-treatment and assessed the relation between under-/overweight and cancer treatments.
Patients and Methods: Inclusion criteria: treatment with anthracyclines (A+/A−), platinum (P+/P−) and/or radiotherapy; age at diagnosis $\leqslant$21 yrs; current age $\geqslant$18 yrs; no evidence of disease and $\geqslant$5 yrs post-treatment. Heights and weights were collected at 4−6 yrs (T5), 9−11 yrs (T10) and $\geqslant$15 yrs post-treatment ($T \geqslant 15$). In adults, BMI < 18.5 kg/m^2 was defined as underweight and BMI $\geqslant$ 25 kg/m^2 as overweight. For children, BMI-equivalents were used according to Cole et al (2000) and Van Buuren et al (2004). Logistic regression was used to study the relation between under-/overweight and the several treatment modalities with adjustment for age at diagnosis.
Results: The inclusion criteria were met by 372 CCS (212 males). Median (range) age at diagnosis was 9.3 (0−21.3) yrs.
At T5 ($n = 301$; age 15.2±4.9 yrs), underweight was found more frequently in A+ CCS versus A− CCS (OR 2.30; CI 1.03−5.16), especially if also treated with alkylating agents (AA) (OR 3.11; 1.05−9.26).
At T10 ($n = 250$; age 19.6±4.7 yrs), the risk of underweight in A+ was not different from A−, however in A+ the effect was dose-related: OR 1.43 (1.07−1.92) per 100 mg/m^2 increase. CCS with cranial or craniospinal radiotherapy (CRT) had less frequently underweight versus CRT− CCS (OR 0.46; 0.21−0.99), but after CRT and steroids the risk of overweight was increased (OR 2.82; 1.17−6.80).
At $T \geqslant 15$ ($n = 198$; age 28.7±6.9 yrs), the risk of underweight in A+ CCS was increased after A$\geqslant$300 mg/m^2 (OR 3.73; 1.11−12.57) or after A in combination with AA (OR 10.72; 1.33−86.46). The risk of overweight was increased in CCS who received CRT (OR 2.83; 1.50−5.34) and in CCS who received CRT in combination with steroids (OR 4.45; 2.01−9.86).
At T5, T10 and T$\geqslant$15, P+ CCS had no more under-7sol;overweight versus P− CCS.
Conclusions: In a CCS-cohort treated with potential cardiac and vascular toxic treatment, A+ CCS were at increased risk of underweight, especially if A dose was higher and if treatment was combined with AA. Furthermore, CRT+ CCS were at increased risk of overweight, especially if CRT was combined with steroids.

1403 ORAL

Fenretinide enhances the antitumour efficacy of bortezomib on human neuroblastoma cells

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Background: Neuroblastoma (NB) is the most common and deadly extracranial solid tumour of childhood and so far is still refractory to conventional therapy. The proteasome inhibitor bortezomib was able to inhibit cell growth and angiogenesis in neuroblastoma (Brignole et al., JNCI 98:16, 2006). Moreover, bortezomib has been shown to induce additive or synergistic activity when combined with several other antineoplastic agents. Here, we assayed a putative increased antitumour activity of bortezomib if delivered to NB cells together with fenretinide, a synthetic retinoic acid used as potential therapeutic agent in a variety of cancers, including NB.
Materials and Methods: Different NB cell lines were tested for sensitivity to bortezomib and fenretinide, when both of the drugs were given to the cells alone or in different dose-and time-dependent combination schedules. Cell proliferation, cell viability and apoptosis were evaluated by measuring 3H-thymidine incorporation, trypan blue staining, DNA fragmentation and western-blot analysis. A mouse xenograft model that mimics the growth and spread of NB in humans was set up to examine in vivo sensitivity of NB to bortezomib and fenretinide. Histologic analysis of mouse orthotopic tumours was performed.
Results: A short (1−4 hours) pre-incubation with 2.5 μM fenretinide caused >50% inhibition of cell growth when NB cells were treated with 5nM bortezomib at 24 hours: these values correspond to at least the half concentration necessary to have the same results when both drugs were administred alone. These results were not obtained when bortezomib was administred to NB cells at the same time or before fenretinide. Preliminary experiments seem to suggest that NB cell death, triggered by the combination of the two drugs, occurs with apoptosis features via ER stress and suppression of the unfolded protein response, that is translated in the activation of different genes, related to these precesses. Furthermore, mice treated with fenretinide followed by bortezomib lived statistically significantly longer than either control mice or mice treated with each single drug.
Conclusions: Our findings provide the rationale for design a new therapeutic strategy to treat pediatric neuroblastoma, based on this pharmacological combination.

1404 ORAL

Proton beam therapy for children with sarcomas: The University of Tsukuba experience

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Background: Proton beam therapy has an excellent dose-localization capability because the beams have a finite range of penetration. It is expected to reduce treatment-related morbidities especially for children who are growing. We reviewed children with various sarcomas irradiated with proton beams to examine its feasibility and efficacy.
Methods and Materials: Between 1984 and 2005, 31 children with typical childhood sarcomas, aged 1 to 15 years, were treated with proton beam therapy at University of Tsukuba. There were 17 boys and 14 girls. Of the 31 children, 13 had rhabdomyosarcoma, 4 PNET, 3 neuroblastoma, 3 hepatoblastoma, 2 osteosarcoma, and 6 miscellaneous. All 13 children with rhabdomyosarcoma had IRS Group III tumors. Sites of irradiation were head and neck for 21 children, abdomen and pelvis for 6, and miscellaneous for 4. Thirty-one children also received chemotherapy prior to proton beam therapy. Of the 31 children, 1 had osteosarcoma of the left arm for which 100 Gy of proton dose in 5 fractions was given. For another 26 children, median 45 Gy (range, 18−73.2 Gy) in median 35 days with median daily dose being 2.0 Gy (1.8−4.2 Gy) were given using proton beams alone. Remaining 5 children were irradiated with a combination of

protons and x-rays: x-ray dose of median 12.0 Gy (6.0−30 Gy) with daily dose of 1.8−2.0 Gy and proton dose of median 38 Gy (30.0−45.2 Gy) with daily dose of 1.8−2.5 Gy in median 36 days were given. Nine children were treated on sedation; No child needed general anesthesia.
Results: No child had to discontinue proton beam therapy, due to treatment. Acute morbidity according to the EORTC scoring criteria was grade 0 for 2 children, 1 for 26 and 2 for 3. Follow-up periods ranged from 8 months to 22 years with a median of 45 months. Over-all and disease-free survival for the 31 children was 61% and 53% at 10 years, respectively. Local control rate in the irradiated sites of the 31 children was 87% at 10 years. For 29 children who were followed for 6 months or longer, late morbidity according to the EORTC scoring criteria in the bone was grade 0 for 25 children, grade 1 for 1 and grade 2 for 3. There was no another serious late morbidity recorded.
Conclusion: Proton beam therapy is at least as feasible and effective as x-ray therapy is. Treatment related morbidity might be less than that of x-ray therapy. Further studies are needed to define the role of proton beam therapy in the treatment of children with sarcomas.

1405 ORAL

Health-related quality of life in long-term survivors of childhood brain tumours

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Purpose: To analyse the impact of potential predictors [gender, age at the time of diagnosis, tumour location, the presence or absence of hydrocephalus requiring shunt inserted at diagnosis, and treatment with radiotherapy (RT)] on health-related quality of life (HRQL) in an unselected population of survivors of childhood brain tumours, and to examine the relationship between cognitive function and HRQL.
Methods: We analysed a consecutive sample of 126 patients [7.9−40.4 years] who had a brain tumour diagnosed before the age of 15 years and were treated during the period January 1970 through February 1997 in the eastern part of Denmark. Sixty-nine had received radiotherapy (RT). In addition to assessment of general intelligence (IQ), an early version of the Minneapolis-Manchester Quality of Life (MMQL) questionnaire was administered.
Results: In multiple linear regression, treatment with RT was the most important risk factor for reduced HRQL. RT showed significantly negative associations with physical functioning, physical energy, body image, social functioning, intimate relations, and outlook of life. Tumour location in the cerebral hemisphere was associated with a less positive body image and older age at diagnosis with better social functioning and relations to the opposite sex. When IQ was included as a covariate, RT only remained significant for social functioning while hemisphere tumour location remained significant for body image, and age of diagnosis for social functioning.
Conclusions: The results suggest that IQ is a very sensitive measure of the effects of brain tumour and a strong determinant of health-related quality of life.

1406 ORAL

Parotid gland sparing in paediatric patients receiving radiotherapy for infratentorial tumours: Optimization of treatment technique to improve normal tissue sparing

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Background: Radiation damage to the parotid glands can be irreversible, and studies of adult patient population have shown that an improvement in some measures of quality of life is achievable with salivary-gland-sparing intensity modulated radiotherapy (IMRT). Avoidance of xerostomia to maintain healthy dentition is especially important in the paediatric population. The use of IMRT is increasing for paediatric brain tumours. Minimizing radiation toxicity to the parotid glands should be included as a priority in planning, particularly for those tumours arising in the infratentorium, in addition to maintaining dose tolerances for other organs-at-risk (OAR), such as optic structures and brainstem. Radiation oncologists and medical dosimetrists are adapting their treatment planning practices towards this end. An average radiation dose of $\leqslant$2600 cGy was found to be the threshold for preserved salivary flow. Three-dimensional radiation dosimetry using non-coplanar beam arrangements could assist with the objective of reducing radiation dose to the parotid glands.
Methods: Computed tomography (CT) and magnetic resonance imaging (MRI) datasets of paediatric patients diagnosed with medulloblastoma or ependymoma were retrieved from the radiotherapy planning archives. Bilateral parotid glands were contoured by the medical dosimetrist and the radiation oncologist using ADAC Pinnacle software. Dose constraints for parotids consistent with those utilised within head and neck radiotherapy planning were applied. Dose calculations were repeated for the purpose of evaluating the dose delivered by conventional and intensity-modulated treatment plans. The gantry angles chosen for target coverage were examined retrospectively, and new non-coplanar beam orientations were employed with the aim to avoid beam entry through the parotid glands.
Results: Dose distributions of an initial six treated plans were compared against the plans generated using non-coplanar beam orientation. Percentage difference of dose to OARs was measured and then averaged. Evaluation of dose-volume histograms and radiation dose statistics demonstrated that non-coplanar beam dosimetry resulted in lower dose to the parotid glands and other OARs. On average, the non-coplanar plans resulted in a decrease of the maximum (−23.1% [right], −23.6% [left]) and mean dose (−16.6% [right], −16.1% [left]) for both parotid glands. The average dose to parotids was within threshold toxicity of $\leqslant$2600 cGy (2314.2 [right], 2294.9 [left]), while maintaining dose limits to the optic structures and brainstem. Further analysis of an additional fourteen patients is ongoing.
Conclusion: IMRT techniques introduce radiation dose to normal tissue surrounding target volumes by the gradient effect; hence, it is important to consider beam orientation when applying it to radiotherapy treatments, especially for paediatric patients.

1407 ORAL

Successful treatment of childhood brainstem gliomas with cisplatin and irinotecan

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Background: Childhood brainstem gliomas (BSGs) are a heterogeneous group of neoplasms with dissimilar natural histories. Historically, the prognosis of BSGs has been exceedingly poor, median survival 4−15 months. No standard chemotherapy has shown a significant impact on BSG outcome, particularly the diffuse intrinsic (median time to progression, 6 months). A pilot study suggested that irinotecan/cisplatin (I/C) is effective for spinal cord astrocytomas (Mora et al, Neuro-Oncol 2007), thus in November 2005 a phase II I/C trial for all progressing astrocytomas was initiated. Here, we focus on the brainstem subgroup.
Materials: From January 2002 to December 2006, 77 patients have been managed for astrocytoma in our institution. Twenty-two (28%) were BSG, 16 managed prior to the I/C protocol, the historical cohort. The indication for adjuvant therapy was based upon histology, surgical resection, or clinical symptoms. Weekly Irinotecan (50 mg/m^2 and 65 mg/m^2 the last 2 cycles) and Cisplatin (30 mg/m^2) for four consecutive weeks (1 cycle), and a total of 4 cycles was used ambulatory. The diffuse intrinsic and high-grade tumors also received antiangiogenic therapy with bevacizumab (5−10 mg/kg, biweekly) and radiation therapy.
Results: Primary sites of the 22 BSGs include: 7 (32%) midbrain/thalamus; 15 (68%) pontine tumors (7 nondiffuse and 6 intrinsic diffuse); and 2 (9%) cervicomedullary. Histology is available for 15 tumors, 4 (26%) being high-grade's (3 pontine and one thalamic) and 11 (74%) low-grade's (4 pilocytic). No patient had clinical signs of neurofibromatosis. Of the 6 patients enrolled, 4 had pontine tumors (2 nondiffuse and 2 diffuse) and 2 low-grade, midbrain tumors. All pts had complete and rapid clinical responses to the I/C regimen. Remarkably, a >20% reduction of the tumor size was achieved with the I/C treatment at the end of therapy, including the 2 intrinsic diffuse BSGs. One midbrain, low-grade tumor has progressed requiring further therapy. All 6 pts are alive and well, median f/u 9 months. In the historical cohort, 10 (62%) pts are alive, median f/u 25 months.
Conclusions: Remarkable early clinicoradiological responses were obtained using the I/C regimen for childhood BSGs.