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