process such as VEGF, bFGF, COX 2, and a relatively high level of DNA topoisomerase II a therapeutic target of etoposide. All microrarray experiments were repeated twice. The high levels of growth factors and COX2 mRNAs were confirmed by immunoistochemistry analysis. On the basis of these results we preceded to antiangiogenetic approach. At this time after few months of follow up the instrumental examinations confirmed a disease stabilization in three patients, a light regression in a patient, while the rapid and extensive progression of the disease caused the death of a child after a month of antiangiogenetic therapy.
Thus, we showed that the gene expression monitoring could provide new insight into many aspects of posterior fossa tumors as revealing targets for antiangiogenetic therapy. New drug development and evaluation will likely be accelerated both through the identification of novel molecular targets and through the selection of patients for clinical trials with specific tumor gene expression profile.

1245 POSTER

Experience with treatment of lymphocyte predominance Hodgkin's disease in children

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Background: We retrospectively evaluate clinical characteristics and outcome in children with nodular lymphocyte predominant Hodgkin disease (LPHD), which is a rare entity characterized by neoplastic popcorn cells CD 20+, CD 30−, CD 15−, EMA+, Bc 16+ within nodular background composed of small B lymphocytes.
Material and methods: From January 1996 to December 2004, 155 children and adolescents with Hodgkin disease were treated in the Department of Paediatric Hematology and Oncology of the Faculty Hospital Motol in Prague. Nodular lymphocyte predominant Hodgkin disease was histologically confirmed in 7 children (4.5%) – 6 boys, 1 girl. The age range was 7–17 years (mean of 14.9 years). Initial staging included complete physical examination, blood studies and imaging studies as X rays, CT scans and 4 patients (57%) had PET scan. Disease presentation was localized in 5 patients (71%) and advanced in two patients (29%) – both Stage III. Only one patient presented with B symptoms and one patient had bulky disease. Neck was the common site of involvement (5 patients). All patients were treated with chemotherapy combined with involved field radiotherapy. Chemotherapy treatment was not uniform – 3 patients received 5 cycles DBVE-PC (doxorubicin, bleomycin, vinkristine, etoposid, prednisone, cyclofosfamide), two patients received 2 cycles DBVE, one patient 4 cycles DBVE due to partial response after the first two cycles and one was treated with 4 cycles ABVD/COPP. Involved field radiation therapy was administered to all patiens in dose 21–25.5 Gy.
Results: All patients achieved complete remission after combined modality treatment. At a median follow up of 3.2 years (range 2.2 to 9 years) 2 patients relapsed (29%). Both relapses were more than 1 year after primary diagnosis (20 and 28 months) and both patients achieved second complete remission.
Conclusion: Current strategy of treatment LPHD is aimed at high cure rates with less toxic regimens to limit risk of late complications and secondary malignancies. But careful long term follow-up is essential for risk of late relapses.
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1246 POSTER

Phase II study of Gemcitabine in children with solid tumors of mesenchymal and embryonal origin

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Background: Gemcitabine inhibits DNA synthesis and repair and shows efficacy in many types of adult malignancies, including previously untreatable pancreatic cancer. No data are available about its effectiveness in children. To determine the efficacy of gemcitabine the drug was administred by i.v. short term infusion over 30 min at a dose of 1200 mg/m^2 weekly for 3 weeks in children with first or subsequent recurrence of a solid tumor of embryonic or mesenchymal origin if standard therapy failed to offer any curative therapeutic option.
Results: From May 2003 to April 2005, 14 male and 6 female patients at the median age of 15.8 years (2–23) were recruited for the prospective open-label multicenter phase II study of gemcitabine in Germany and Austria. The patients suffered from soft tissue sarcoma (n = 8), Ewing's sarcoma (n = 4), Neuroblastoma (n = 3), Hepatoblastoma (n = 2), Osteosarcoma (n = 2) or Nephroblastoma (n = 1). Mean duration of therapy was 31.4 days (7–99), equalling 4.6 (2–11) courses of gemcitabine. 2 patients, whose "Best Overall Response" according to RECIST-criteria (i.e. minimal 6 courses) was evaluable, had stable disease documented for 69 and 70 days, respectively (neuroblastoma, Ewing's sarcoma), whereas no response to gemcitabine was documented. The other patients left the trial mainly due to early progress. The mean dosage per course was 1104 mg/m^2. In 33/88 evaluable courses dosage had to be reduced or omitted for grade 3–4 haematologic toxicity. No suspected unexpected serious adverse reactions (SUSAR's) were reported.
Conclusions: Gemcitabine at the dose and schedule of this trial was not effective for children with refractory solid tumors. Given the variety of other promising agents, further evaluation of gemcitabine as single treatment of childhood solid tumors does not appear to be warranted. Nevertheless, publishing of negative results is indispensable for diminishing the Publication only bias.

1247 POSTER

Alterations of brain metabolism after therapy of paediatric brain tumors. a serial proton magnetic resonance spectroscopy study

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Aim: The aim of the study was to evaluate metabolic changes in tumor bed occurring after therapy of pediatric brain tumors using serial proton magnetic resonance spectroscopy studies.
Material: The examined group consisted of 15 children with brain tumors treated with surgery, chemotherapy and radiotherapy. Eight children had medulloblastomas, four had astrocytomas, one had oligodendroglioma, one glioblastoma and one had a mixed tumor having features of both PNET and glioblastoma.
Methods: Short echo-time (TE 30 ms) point-resolved spectra were acquired using 2 Tesla clinical scanner (Elscint Prestige). The proportions of N-acetylaspartate (NAA), choline (Cho), myo-inositol (mI), lactate (Lac) and lipids (Lip) signal intensities were calculated using creatine (Cr) signal as an internal reference. The spectra were acquired from tumor bed and form unaffected brain tissue of contralateral hemisphere as a comparison. The first examination was made between third and sixth month after therapy, the second 8–12 months after therapy and the third examination was performed approximately 18 months after completion of therapy. The results were compared using t-test for dependent samples.
Results: In all cases there were significant disturbances in brain metabolism detected both in the spectra acquired from the tumor bed and from control area. The most important alterations were decrease of NAA/Cr and increase of Cho/Cr, Lac/Cr and Lip/Cr proportions. The observed changes did not differ significantly between subsequent examinations.
Conclusions: Alterations of brain metabolism after combined therapy of brain tumors in children affect both tumor bed and uninvolved area of brain tissue and are stable in subsequent examinations indicating long-lasting or permanent brain damage.

1248 POSTER

Expression profiles of 'minimal residual disease' (MRD) markers for neuroblastoma in peripheral blood and its cell fractions by real-time quantitative PCR (RQ-PCR)

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Background and Aim: In neuroblastoma (NBL) specific and sensitive markers are essential for MRD detection. NBL cells highly express tyrosine hydroxylase (TH), DOPA decarboxylase (DDC), chromogranin-B (CHGB), GD2 synthase (GD2), dopamin beta hydroxylase (DBH), paired-like homeobox 2B (PHOX2B), growth associated protein 43 (GAP43), synaptosomal associated protein (SNAP), stathmin-like 2 (STMN2), stathmin-like 4 (STMN4), and cholinergic receptor (CHRNA3). TH, DDC, GD2 and CHG are among common MRD markers for NBL. In previous experiments, PHOX2B had no expression in normal bone marrow (BM), DDC was expressed in 2/67, and other markers in $\geqslant$ 14/67 samples. In peripheral blood (PB), PHOX2B, DDC, CHRNA3 were negative; TH was positive in 2/22, DBH in 1/22 samples, and others in $\geqslant$ 10/22 of samples.

We aimed to determine the expression profiles of MRD markers in PB cell fractions and in PB stem cells (PBSCs) by RQ-PCR to determine cellular origins for illegitimate expressions.
Materials and methods: Granulocytes (gran.), T and B cells, monocytes (mono.), NK cells, and platelets (plts) from PB of healthy donors, and also samples of PBSCs were collected. RQ-PCR assays were based upon TaqMan technology using specific primers and probes. Total RNA was isolated and RNA samples were reverse transcribed by standard methods. Then, 50 PCR cycles were performed by ABI Prism 7700 sequence detector. All samples were studied in duplicates. Beta glucuronidase (GUS) was used as the 'housekeeping gene'. IMR-32 NBL cell line was examined as positive control. Samples with CT values > 39.5 were accepted as negative. Results were compared to CT of GUS and ΔCT values ($CT_{GUS}-CT_{Marker}$) were calculated.
Results: PHOX2B was negative in all PB cell fractions. Number of positive samples for each marker in different cell fractions were: DDC 1/12 gran., 1/10 mono.; DBH 1/10 T cells; CHRNA3, 3/10 mono., 2/10 T cells; TH 5/12 gran., 8/10 mono., 3/6 NK cells; CHGB 11/12 gran., all T cells, 2/10 B cells, 4/10 mono., 3/10 plts; GAP43 1/12 gran., 1/10 T cells, all B cells, 1/10 mono., 2/10 plts. GD2, SNAP, STMN2 and STMN4 had varying degrees of positivity in all cell fractions. In PBSCs PHOX2B, DDC and CHRNA3 were negative, DBH was positive in 1/10, and others in ⩾ 8/10.
Conclusions: PHOX2B is the best marker for MRD detection in NBL. In BM DDC, and in PB and PBSCs DDC, DBH and CHRNA3 also appear to be useful MRD markers. These results will guide future studies for more sensitive detection of MRD in NBL.

1249 POSTER

Could angiogenesis and the expression of Ki-67, p-53, p-27 and bcl-2 be prognostic factors in highly malignant brain tumors of childhood?

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Background: P-53, p-27 and bcl-2 are proteins encoded by genes that are implicated in cell cycle control, as well as in angiogenesis. Ki-67 is an index of cell proliferation. Aim of this study is the assessment of angiogenesis in highly malignant brain tumors in relation to the expression of Ki-67, bcl-2, p-53 and p-27. In addition, we investigated the possible association of these factors with the prognosis of the disease.
Material and methods: 29 children with embryonic type brain tumors enrolled in the study – 24 patients with Meduloblastoma (MB) and 5 with Atypical Teratoid/Rhabdoid Tumor (ATRT). The Streptavidin-Biotin immunohistochemical method was performed on tissue paraffin sections. The CD31 (YLEM®) monoclonal antibody was used in order to detect new blood vessels and determine their density. Monoclonal antibodies were also used for the detection of Ki-67 (DACO®), bcl-2 (Novocastra®), p-53 (DACO®) and p-27 (Novocastra®). All patients were followed up for 2 to 8 years (mean follow-up time: 4.7 years).
Results: Newly formed blood vessels were detected in all cases, whereas high density of new vessels was established in 12 MB and 3 ATRT. High levels of Ki-67 index (>25%) were detected in 87% of MBs. MBs with high density of new vessels presented with concomitant increased expression of Ki-67. The majority of MBs (79%) had high expression of p-27. P-53 was detected in 75% of MBs while bcl-2 in 20% of MBs. No correlation was found between the expression of p-53 or bcl-2 and the histological type of tumor. High density of newly formed vessels in association with high expression of Ki-67 and p-53 was detected in all ATRTs. High expression of bcl-2 and p-27 was detected in the majority of ATRTs. Eight patients died (five of the MBs and three of the ATRTs). In all deceased patients, high levels of angiogenesis in association with high expression of both Ki-67 and p-53 was observed.
Conclusions: Increased angiogenesis seems to be associated with high expression of Ki-67, p-53 and p-27. Angiogenesis along with high levels of Ki-67 and p-53, could possibly have a prognostic value for the disease outcome. Study of the above indices at diagnosis could alter or intensify treatment protocols, in order to improve the disease outcome.

Publication
Paediatric oncology

1250 PUBLICATION

Significance of routine bone marrow, CBC and physical examination for early diagnosis and outcome of relapse

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Children with Acute Lymphoblastic Leukemia (ALL) treated according to BFM 90 and 95 in our department after remission induction are routinely evaluated as follows: Bone marrow (BM) four months (mo) from diagnosis (dx) and one mo after therapy completion, complete blood count (CBC) approximately 101 (77 during maintenance and 24 the first 4 years off therapy) and physical exam (PE) approximately 42 (18 monthly during maintenance and 24 while off therapy). Of 50 relapses documented during the study period, 24 – group A – (22 BM, 2 testicular) were diagnosed during routine evaluation (BM 6, CBC 16, PE 2). Group B included 26 symptomatic relapses (fever, echymoses, lymphadenopathy, abdominal pain, headache, vomiting, vision problems), 11 in BM, 6 in central nervous system (CNS), 3 testicular, and combined 6 (BM+CNS 3, BM+testis 3).
Second complete remission (CR) was obtained in 15/24 (62.5%) of group A and in 20/26 (76.9%) of group B. Overall survival (OS) in group A was 29.2% (7/24) for 28–94 mo from relapse (med 53) in CR2 6 and in CR3 1. OS in group B was 38.5% (10/26) for 5–108 mo from relapse (med 25) all in CR2. Of 388 routine BM 6 were positive (0.015%), of almost 20,000 CBC 16 were positive (0.0008%) and of almost 8000 PE 2 were suspicious (0.00025%).
From this retrospective study it is shown that routine evaluation in children with ALL may not depict relapse and therefore may not influence final outcome despite the fact that the number of relapses which we found is significant (24 versus 26).

1251 PUBLICATION

Preliminary experience with intensity modulated radiotherapy (IMRT) in the management of pediatric brain tumors (PBT)

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Introduction: radiotherapy (rt) is a cornerstone in the management of PBTS. Unfortunately its use has been associated with multiple deleterious side-effects esp in young children, that can affect cognitive, pituitary, auditory, visual. functions, and with carcinogenic effects. New technologies, like conformal radiotherapy or IMRT, are considerably promising, but their respective merits rarely quantified.
Patients and methods: Four PBTS in children > 6 years, located in the supra tentorial region, were treated using IMRT: superficial cortex (resected ependymoma), deep-sites (craniopharyngioma, optic glioma), and extended complex target (ventricular rt in dysgerminoma). Total dose = 24–55 Gy. 3D virtual simulation was performed based on high definition fused CT/MRI imagings. Target and safety margins were delineated and 3D planning generated (Helios-Cadplan dynamic therapy). Treatment was performed with a Varian machine (52 multileaf collimator). For the purpose of the study, a dosimetric intercomparison with usual conformal rt was made based on dose volume histograms (DVH). Emphasis was put on tight conformation using Conformity Index (CI) to Planning Treatment Volume (PTV), and sparing of critical structures.
Results: Clinical evaluation of the 4 pts showed an acceptable acute tolerance, although close to usual techniques. With a 9–18 months follow-up, all pts have remained with NED, and no complications. Dosimetric evaluation of the IMRT plans, evidenced improved conformation of the 95%, and uniformity of the 100% isodoses to target, especially in case of complex target shapes (ventricular rt: CI: 1.25 vs 2.67). Conversely, hot spots were increased by 5–10%, in most situations. Doses to critical organs were generally lowered with IMRT, although to a moderate extent, and no substantial increase of the integral dose to surrounding structures was recorded.
Conclusion: IMRT is feasible in children and well tolerated. An ultimate conformation to high doses is made possible, especially in case of complex target shapes. No increase of the integral dose to surrounding anatomical structures was observed which tends to indicate that the carcinogenic risk was not impacted negatively.