Akt3 in these cell lines. This increased activity also correlated with faster proliferation rates and increased survival in the absence of serum. Treatment of the cells with the PI3K inhibitor LY294002 resulted in increased cell death in those cells with high Akt activity. Akt isoform expression was assessed in 53 primary ovarian tumour samples revealing high Akt3 expression in 33% mucinous, 59% serous, and 66.6% endometrioid tumours. High Akt2 expression was observed in 11% mucinous, 18% serous but not in endometrioid tumours. Positive phospho-S473 staining, representing active Akt, correlated with high Akt3 expression.

**Conclusion:** These results suggest that Akt3 may play an important role in ovarian tumourigenesis.

634 POSTER

### The cytochrome p450 (CYP) family 1 at early stages of carcinogenesis.

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**Background:** CYP1 plays an important role in activation of environmental carcinogens including polycyclic aromatic hydrocarbons, PAH and some drugs. It was suggested that PAH is present in tobacco smoke and participates in tumor development in smokers. There are many studies of CYPs in cancers, however the data on regulation of CYP isoforms at early stages of carcinogenesis are limited. Using two models of cell immortalization and transformation, we studied mRNA expression and activity of CYP1 enzymes as well as the expression of *AHR* and *ARNT* genes that encode the regulators of CYP1 expression.

**Material and methods:** cultivation of cell culture, RNA isolation, RT-PCR, benzo/a/pyrene-hydroxilase activity assay, cell transfection, cell survival assay.

**Result:** In the embryo rat fibroblasts (RF) constitutive level of *CYP 1A1* mRNA was not detectable, whereas *CYP1B1* mRNA was expressed. After cells immortalization with Rauscher virus (F-27/RLV), mRNA level of *CYP 1A1* became high, and *CYP 1B1* level increased in comparison with RF cells. The F-27/RLV cells oxidized benzo/a/pyrene more effectively and were more sensitive to toxic effects of benzo/a/pyrene and 7.12-dimethylbenz/a/anthracene than RF cells. In spontaneously immortalized embryonic rat fibroblasts (Rat1) we found high expression of *CYP1B1* mRNA compared to RF cells. Treatment with TCDD increased *CYP 1B1* mRNA level in both rat cell lines. Unlike RF, Rauscher immortalized cells with relatively high level of CYP1 expression were sensitive to transforming effect of benzo/a/pyrene. In transformed clones levels of *CYP1A1* and *CYP1B1* mRNA were lower compared with F-27/RLV cells. Benzo/a/pyrene - hydroxylase activity decreased in transformed cells. In Rat/ras transformed cells obtained after transfection of *N-ras*$^{asp12}$ gene, the constitutive expression of *CYP1B1* mRNA disappeared in comparison with Rat1 cells. The mRNAs of proteins which take part in the regulation of enzymatic induction of CYP1 (AHR and ARNT) were the same in all cell models studied.

**Conclusion:** Constitutive and inducible levels of *CYP1B1* mRNA increase after immortalization. Transformation of immortalized cells provokes disappearance of *CYP1B1* expression. Since *AHR* and *ARNT* expression are similar in all cells studied, we suggest that other factors besides AHR and ARNT take part in CYP1 regulation.

## Paediatric oncology

635 POSTER

### Second tumors after treatment for Hodgkin's lymphoma (HL) in children

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Second malignant tumors (SMT) are one of the most severe complications in children treated for HL. The aim of the study was to investigate the incidence of second tumors in children with HL.

**Materials and methods:** We observed 450 pts. in CR after treatment of HL from 1972 to 1997. The period after treatment was 60-360 months (med.=134,5). The age of pts was from 2 to 18 years (med.=12,5 yrs; M/F=2,6/1). Histological subtypes nodular sclerosing and mixed cellularity were predominance. The III and IV stages of HL were reveal in 70% patients. Practically all children were undergo combined treatment HL, which were include chemotherapy (MOPP, COPP, ABVD, OPPA, PCVP) and radiotherapy (20-50 Gy).

**Results:** Second tumors were found in 20 pts (4,4%). Malignant tumors were in 12 pts (2,7%), and benignant tumors 8 pts (1,8%). Among SMT were: stomack cancer -3, breast cancer -2, thyroid cancer -1, liposarcoma -1, glyosarcoma -1, malignant schwannoma -1, rhabdomyosarcoma -1, acute myeloblastic leukemia -1. SMT were reveal in period from 30 to 340 months to beginning the treatment HL (med. =165 months). Mortality rate for patients with SMT compose 41,7%. Second benignant tumors (SBT) were diagnosed in 8 pts during 46-300 months (med.=153). Thyroid adenoma were in 5 pts, breast fibroadenoma 2, papillomatosis of larynx 1. One patient suffered from 2 SBT thyroid adenoma and neurinoma.

**Conclusion:** SMT more often reveal in patients with HL, which treated in age older 10 years (8 pts from 12) and in women (8 pts from 12). All the second solid tumors (SMT and SBT) are localized in zone of radiotherapy with dose 40 Gy. Thyroid and breast tumors are the most frequent in structure of second tumors.

636 POSTER

### Neuroblastoma in adolescents and adults: analysis of a mono-institutional series of 33 consecutive patients.

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**Background:** Neuroblastoma (NBL) is the most common extra-cranial solid tumor in children. More than 95% of patients at diagnosis are younger than 10 years. Adolescents and adults have a grave prognosis, but may have a more indolent course.

**Material and methods:** From 1980 to 2002, 33 patients (stage I= 3, stage II= 6, stage III=8, stage IV=16) with newly diagnosed NBL older than 12 have been admitted at Istituto Nazionale dei Tumori of Milan. Median age was 17 yrs (range 12-69); M/F ratio was 1.2. Symptoms were present and disregarded for many months in the majority of cases: the mean time frame between the onset of symptoms and diagnosis was 15 months. Site of the primary tumor was retroperitoneum in 19 cases, mediastinum in 5, pelvis in 1, cervical in 1, while 6 had an esthesioneuroblastoma; 1 case the primary tumor was unknown. LDH was elevated in 15/33 pts. *Treatment applied*: surgery alone for stage I; post-operative radiotherapy for stage II; stage III and IV received chemotherapy regimens including anthracycline + cyclophosphamide + vincristine ± ifosfamide ± etoposide± cisplatinum. In addition, 10/16 stage IV pts were submitted to sequential hemi-body irradiation as consolidation treatment. Radiotherapy and/or surgery on primary and metastases were decided on individual basis.

**Results:** 20/33 relapsed: 0 stage I, 1 stage II, 4 stage III, 13 stage IV, and 18 relapsed died. The median follow-up is 42 months (range 12-264). EFS and OS probability at 5 years are shown in the table:

| | Stage I | Stage II | Stage III | Stage IV |
|---|---|---|---|---|
| EFS | 1 | 0.67 | 0.40 | 0 |
| OS | 1 | 0.83 | 0.56 | 0.12 |

In this series a consistent number of late relapse/progression were observed: time to progression/relapse ranged from 3 to 58 months and the time from relapse to death from 2 to 75 months. In univariate analysis, together with the stage, the only statistically significant prognostic factor is LDH level at diagnosis: an elevated LDH negatively predicted the outcome (5 yrs OS: normal 54%, pathological 0; p 0.0089).

**Conclusions:** Localized NBL (stage I and II) in adolescents and adults have the same good prognosis of children. For pts with locally advanced and metastatic disease, late events were frequently observed, thus suggesting a lower biological aggressiveness of the disease in this subset. Nevertheless, the prognosis for these patients is dismal.

637 POSTER

### Evidence for a redox mechanism of action of prednisolone in childhood acute lymphoblastic leukaemia through the identification of the novel gene CGI-31.

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Glucocorticoids are the most important drugs used in the treatment of acute lymphoblastic leukaemia and poor response to these drugs during

induction is a powerful adverse prognostic factor. However, the mechanisms by which glucocorticoids induce cytotoxicity are poorly understood. Using the T-lymphoblastic cell line CCRF CEM C7, we have demonstrated the involvement of a novel gene with proposed thioredoxin function in the response to the glucocorticoid, prednisolone.

Global gene expression profiles were examined in sensitive and resistant populations of CCRF CEM C7 using the technique of differential display. Quantitative RT-PCR was used to confirm altered gene expression.

Using differential display, apparent down-regulation of the novel gene CGI-31 was seen in sensitive but not resistant leukaemia cells during 6 hours of prednisolone exposure and this was confirmed using quantitative RT-PCR.

638 POSTER

## Pharmacodynamics of aplidinR (APL) in experimental models of haematological malignancies (HAMA)

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APL is a marine derived COMPARE negative cyclodepsipeptide isolated from the tunicate *A. albicans*. The phase I program has been completed being muscular tox the dose limiting factor with lack of bone marrow suppression; In contrast APL has been shown to inhibit the VEGF secretion and to block the VEGF/VEGFR1 loop in the acute lymphoblastic leukaemia (ALL) MOLT-4 cells. Such evidence is consistent with the induction of apoptosis and % cell death (median 97%) in ALL *de novo* and relapsed fresh patient's blasts at 0.5nM. Extended studies in ALL and AML pediatric samples have confirmed *in vitro* cytotoxicity at concentrations (CO) achievable below the recommended dose. In contrast supraphamacological COs are needed to induce cytotox against normal bone marrow progenitors and peripheral lymphocytes; Cross resistance studies have failed to demonstrate a pattern of resistance between conventional antileukemic agents and APL. Comparative studies demonstrate that APL is 10 fold more potent than Idarubicin in a panel of AML patient's blasts with respective median IC50s= 0.048uM and 0.357 uM. Moreover, clinically relevant CO of APL are also able to induce cytotoxocity against fresh samples from patients with CLL and against samples from multiple myeloma resistant to dexamethasone. In addition, *in vitro* combination studies in AML, ALL and non-Hodgkin lymphoma indicate statisitcally significant synergistic effects when sub-toxic CO (IC20) of APL are combined with standard agents.

| Additional drug | IC50 DOXO | IC50 MTX | IC50ARA-C |
|---|---|---|---|
| -APL | 18nM | 5nM | 30nM |
| +IC20APL | 1nM | 500pM | 6nM. |

In conclusion the available data with APL, a non myelotoxic drug, indicates selective cytoxity against a set of experimental models of HAMA at COs that are achievable well below the RD. Such evidence supports the clinical development of APL in these settings.

639 POSTER

## Ganglioneuroma in childhood: the Italian experience

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Ganglioneuroma are benign neuroblastic tumours. Few informations about optimal treatment and outcome of this tumour are currently available.

We searched the Italian Neuroblastoma Registry for cases of ganglioneuroma and sent a questionnaire to all Italian Paediatric Oncology Centres. Questions concerned sex, age, symptoms at diagnosis, surgery and follow-up. We also asked the participant Centres to send the surgery description and the histological report of each patient.

Since 1976, January 1 to 2002, December 31 159 cases were diagnosed. 66 were males, 93 females. Median age was 5 years and 11 months (range 0- 14 years and 5 months). Of 141 evaluable cases, 70 had a thoracic tumour, 53 an abdominal one, in 12 the tumour was pelvic and in 6 latero-cervical. In 2 cases the mass had an intraspinal extension.

63 patients were asymptomatic. The most frequent symptom was pain (23 cases), followed by cough (16) and fever (12). In 10 cases the mass was found at a routine physical examination. Interestingly, 4 patients had scoliosis at diagnosis, 5 presented with urinary symptoms (haematuria, disuria), 3 had Claude-Bernard-Horner syndrome as first sign, and 3 had neurological symptoms (paraplegia, neurologic bladder). Information about surgery was available for 144 cases. 130 underwent a radical or partial tumour excision. In 14 cases only a biopsy was performed at first, and it was followed by radical or partial excision in 8/14 cases. In the remaining 6/14 patients the tumour was not removed and a careful follow-up was started. Early complications of surgery occurred in four cases (pleural effusion, chilothorax, aortic rupture, and mild hischemic suffering of the spinal cord). 13 patients had permanent Claude-Bernard-Horner syndrom after surgery. Nephrectomy was performed in 3 patients, to achieve a complete resection of the tumour. Only one case was treated with chemotherapy and one received radiotherapy (2750 Rad). Median follow-up is 4 years (range 1 month- 15 years, 98 patients available). 103 patients are alive without disease and 15 are alive with stable residual disease. 2 patients underwent disease progression (one had a partial resection at diagnosis, the other had only a biopsy). Both are alive and well after secondary surgery. 4 patients relapsed. All are alive and free of disease after surgery.

Our data demonstrate that ganglioneuroma is a benign disease. The extent of surgery at diagnosis does not correlate with the outcome, although it might be useful to perform at least a partial resection of the tumour, to allow discrimination between ganglioneuroma and nodular ganglioneuroblastoma. Surgery can be difficult and complete resection might require an aggressive approach. We recommend to avoid aggressive surgery, even if complete resection is not otherwise possible.

640 POSTER

## Delay in diagnosis of children with cancer: a retrospective study of 315 children

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**Objectives:** Cancer in children can be difficult to diagnose in the primary setting leading to some delay in diagnosis. Our aim was to determine the demographic and systemic parameters in children with solid tumors and to ascertain which of them affected the delay in diagnosis.

**Methods:** Lag time' was defined as the interval between onset of symptoms and final diagnosis. A retrospective study was performed on 315 children diagnosed with a solid tumor between 1993-2001 at the Department of Hemato-Oncology at Rambam Medical Center. A questionnaire was completed for each child, including epidemiological, social and medical issues concerning the family, the child, the medical system and the tumor. Lag time, including parent delay and physician delay, was estimated for each case.

**Results:** Mean lag time: 15.75 weeks, median: 7 weeks, range: 0-208 weeks. Lowest mean values appeared in kidney tumors, highest values for epithelial tumors, brain tumors and soft tissue sarcomas. Mean parent delay: 4.42 weeks, median: 1 week, range: 0-130 weeks. Mean physician delay: 11.17 weeks, median: 4 weeks, range: 0-206 weeks. One-quarter of patients were diagnosed within 3 weeks, 50% within 7 weeks, and 75% within 15 weeks.

Multi-variant analysis: Five factors were found to be strongly associated with lag time: age of child (older children presented later), ethnic origin of father (greater delay if he was 'Sephardic'), family religion (greater delay in Jews), serial number of the child in the family (greater diagnosis delay in families with one child) and family place of residence (shorter diagnosis delay in the village). Among the demographic and personal parameters, the best predictors for diagnosis delay were age of child and father's ethnic origin.

**Conclusions:** This work demonstrated that there are several factors influencing the diagnosis delay of childhood solid tumors. Recognizing these factors coul d minimize the diagnosis delay, hence improving the chances of the child survive.

641 POSTER

## Seeking for a second opinion in paediatric oncology

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**Objectives:** the number of second opinions consultations in pediatric oncology is increasing, yet the grounds on which families decide to seek a second opinion have been little studied. The goal of the study was to identify