history of orchiopexy. Tumor markers were elevated in 23 pts (34%) after surgery. The histology was combined germ cell tumor in 51 (76%); 20 pts (30%) had seminomatous and 15 (22%) choriocarcinoma components. Of the 44 pts with normal markers after surgery, 21 with no adverse prognostic factors were followed closely on a surveillance protocol. The follow-up included markers every month, chest x-ray every two months and abdomen ct every four months during the first year; markers and chest x-ray every two months and abdomen ct every six months on the second year; markers and chest x-ray every 3 months and abdomen ct every six months on the third year; and six-monthly visits thereafter to complete 5 years. Only one of the pts on surveillance (5%) relapsed and was treated with CT. Of 23 pts who received adjuvant CT (etoposide/cisplatin or bleomycin/etoposide/cisplatin) 2 had relapse. One of them died due to noncompliance, the other died very shortly after early development of massive liver metastases. Of the pts who received CT for elevated markers after surgery, 2 relapsed in the retroperitoneum; both were successfully salvaged by CT +/– retroperitoneal surgery. With a median follow-up of 46 months, median overall survival was 42 months, and the 5 year cumulative survival was 97%. Because of the very few number of events in this good-prognosis group, no difference in survival was detected between the surveillance and adjuvant CT groups. The survival data show that the patient selection for, and the policy of surveillance was justifiable. Randomized trials are needed for prognostic factor analysis in stage I NSTC.

1424 PUBLICATION

Chromosomal aberrations in bilharzial bladder cancer using fish technique

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Cancer of the bladder is a frequent malignancy in Egypt and other developing countries in which bladder infection with the parasite Schistosoma haematobium is common. Several epidemiological, histopathological and clinical characteristics of cancer of the bilharzial bladder suggest that it is distinct from bladder cancer in other places in the world.

No numerical aberration of chromosomes that might be specific for bilharzial bladder carcinoma has so far been established. In this study, we used fluorescence in situ hybridization (FISH) with centromere-specific probes for chromosomes 1–12, 15–18, x and y to detect numerical aberrations of these chromosomes in frozen samples of 31 Egyptian patients affected with bilharzial carcinoma. The most common observed chromosomal imbalance was a loss of chromosome 9 (48.4%), with numerical aberration of chromosomes y and 17 being the second most frequent anomalies (22.2% and 19.4% respectively). The presence of such anomalies especially losses of chromosome 9 are associated with younger age group of patients as well as with lower grade tumor and negative pelvic node affection by the disease.

FISH analysis thus proved to be a useful method for detecting numerical aberrations of individual chromosomes, with application to touch print preparations of frozen – stored tissue having the advantage of exact sampling of cancer foci. This result also suggests that the mechanism of genetic progression of bladder cancer is independent of its etiology.

1425 PUBLICATION

Dose intensity (DI) and total dose (TD) of VAB-6 regimen for metastatic germ-cell tumours

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Purpose: We have analyzed DI and TD in VAB-6 regimen with respect to tumour response and survival.

Patients and Methods: The retrospective study was performed on 70 metastatic germ-cell tumour patients (pts). Pts were given up to six courses of modified VAB-6 as the primary regimen (vinblastin 10 mg, actinomycin D 2 mg, cyclophosphamide 1000 mg D1, bleomycin 10 mg D1–6, cisplatin 50 mg D 7–10).

Results: The complete remission was achieved in 51/71 pts (73%). The overall 5-year survival rate was 68%, and 10-year survival rate was 64%. The average relative dose intensity (ARDI) of planned regimen was 0.75 of standard one. ARDI of applied regimen was 0.90 of planned VAB-6 regimen. The mean value of relative DI (RDI) for cisplatin was 0.88. Average TD/TD of standard regimen ratio for cisplatin was 1.77.

Comparing groups of pts received RDI $\geq$ 0.8 and RDI < 0.8 (for each drug, and for the regimen as a whole), no significant differences were noticed in terms of efficacy and survival.

Conclusion: Two third of pts who are alive 10 years after treatment with greater total dose of cisplatin received permit speculation that TD of cisplatin might influence more the regimen efficacy than ARDI.

1426 PUBLICATION

Gemcitabine (G) and vinorelbine (V) in pretreated or elderly transitional cell carcinoma (TCC) patients (PTS): A phase II study

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The aim of the study was to verify tolerability and efficacy of G and V combination in TCC pts relapsed after platinum-containing regimens (5 pts) or not amenable to platinum because of age > 70 years or poor performance score (5 pts). Mean age was 68.5 (51–78), M/F ratio 2/10; sites of metastases were lung (3 pts), lung and nodes (2), liver and lung (2), pelvis nodes and bone (1); 1 pt had synchronous kidney and lung metastases, 1 pt had locally advanced TCC. 5/10 were platinum pretreated. Mean disease free interval was 8 months (5–45). G (1000 mg/mq/d) and V (25 mg/mw/d) were given on day 1 and 8, every three weeks; no elective g-csf was used. All pts are evaluable for toxicity and response. Fortythree cycles were administered, with a mean number of 4.3 cycle/pt. Grade III–IV toxicities occurred in 1 pt (G IV emesis plus G III neutropenia in a 73 year old women at the 5th cycle, after cPR); other toxicities were G II emesis (5 cases), G I fever (3), AST/ALT elevation (2), cutaneous rush (1). Responses were complete in 2 pts (1 in lung and liver, 8 months duration; 1 in lung, 8+ months); partial in 5 (mean duration 6+ months); 2 stable disease, 1 progression (liver). This treatment seems feasible and active; further studies with larger number of patients are needed.

1427 PUBLICATION

Value of humoral immunity, angiogenesis and basement membrane changes in the prediction and prognosis of bladder carcinoma

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Purpose: The aim of this work was to assess the value of immunoglobulin secreting cells (mediators of humoral immunity), angiogenesis and basement membrane changes in the prediction and prognosis of neoplastic bladder lesions.

Methods: 23 specimens (sp.) of TCC stage pTa, pTis, pT1, 39 sp. of non malignant urothelial abnormalities with atypia either with mild, moderate (26 sp.) or severe (13 sp.) atypia and 7 normal control sp. were subjected to direct immunofluorescence technique using antihuman polyvalent Ig. Positive cells were scored/HPF. Also the sp. were processed for ultrastructural study.

Results: Although a significant increase in the number of Ig secreting cells was elicited in neoplastic and dysplastic bladder lesions versus the benign lesions with mild or moderate atypia ($P < 0.01$), yet no significant difference was detected between the different grades or stages of the studied TCC or between them and the severe dysplastic lesions. On the other hand, the appearance and the increase in the number of abnormally thickened and chained microvasculature just beneath the urothelial BM correlated with the severity of BM involvement in severe atypia and tumor lesions as detected by electron microscopy.

Conclusion: Apart from the detected prognostic value of ultrastructural changes seen in BM of tumor lesions, the appearance of abnormal microvasculature with the occurrence of small vacuoles or irregular thinning in the BM and increase in Ig secreting cells in severe dysplastic lesions may be a predictor factor for a malignant behaviour.

1428 PUBLICATION

Contribution to split-course method in radiotherapeutic treatment for bladder cancer

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Background: Bladder carcinoma is a rare carcinoma relatively, about 5% malign tumor in males and 3% in females. It has been most frequent in the seventh and eighth life decades. In the last decades an increased number

of the diseased with this malign illness has been noticed. The aim of this paper is to evaluate the results of the radiotherapy for bladder carcinoma by Split-course method applied.

Methods: After the schematic treatment we applied individual treatment in that way that we determined the localization and a size of the bladder and localization of the tumor. The radiotherapy was applied according to the following protocol of Split-course method: a total dose of 6000 cGy from the two opposite fixed fields was applied, 3000 cGy in 10 fractions.

Results: There were 148 patients with bladder carcinoma subjected to radiotherapy of Split-course method from 1985 to 1993. If we examine the 5-year survival rate we can conclude that the 5-year survival rate was 87.5% in the first stage. In the second stage even of the total 37 treated survived 5 years, in the third stage 9 lived longer than 5 years out of 21 patients treated if we consider. The total number of patients who survived 5 years we can notice that it is a high number 71 (47.9%) and that the results of our treatment are better than the results found in the world literature.

Conclusion: The contribution of the Split-course method in the treatment of bladder carcinoma resulted is in the reduction of the total time of treatment, the reduced number of fractions and the preservation of radiobiological efficacy.

Biotherapy-gene therapy-vaccination

1429 ORAL

Peptide aptamers: A new generation of molecules for the specific inhibition of oncoproteins?

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Human papillomaviruses (HPVs) are closely associated with the development of several cancers in humans, including cervical cancer. The tumorigenicity of HPVs depends on the expression of the viral E6 and E7 oncogenes. The E6 protein has anti-apoptotic potential and may counteract the elimination of HPV-positive cells under the abnormal growth stimulation by E7. Molecules that can specifically inhibit E6 could therefore form a novel basis for the development of molecular strategies to fight HPV-positive dysplasias and cancers.

The "peptide aptamer system" allows an in vivo selection in yeast for small molecules specifically binding to and functionally inhibiting a given target protein. We here screened a randomized peptide expression library for conformationally restrained 20-mer peptides binding to the human papillomavirus type 16 E6 oncoprotein. We isolated several peptide aptamers that bound with high affinity to the viral oncoprotein in vivo. These interactions were highly specific for E6 and no binding was observed to heterologous control proteins. Some peptides also interacted with E6 proteins of other HPV types, indicating the existence of common E6-epitopes. The peptide aptamers are currently also tested for their effect on E6 activities in mammalian cells and their influence on the tumorigenic phenotype of HPV-positive cancer cells.

Inhibitory peptide aptamers can be used in basic research as experimental tools to investigate the function of the HPV E6 oncoprotein in human tumor cells and, under therapeutic aspects, may serve as lead structures for the development of novel drugs specifically targeting HPV-positive cells. In principle, this approach is also applicable for the identification of low molecular weight inhibitors of any given target protein of pathological significance.

1430 ORAL

In vitro evaluation of a tumor vaccine based on the xenogenization of tumor cells with tetanus toxoid molecules

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The goal of this research project was the design of strategies for anti-tumor immune therapy based on the application of the xenogenization concept. We extended earlier experiments by loading of teanus toxoid, as opposed to peptides comprising xenoepitopes, into human primary tumor cells including primary leukemia cells and culture adapted primary neuroblastoma cells. To mediate loading we used polyarginin (pA) molecules of various degrees of polymerization, cationic liposoms, or chimeric molecules of transferrin (Tf) and the polycation polyethylenimin (PEI). All human primary tumor cells and cell lines studied could be loaded with high efficiency by all procedures as determined by flow cytometric detection of fluorescein labeled TT. Trypsin treatment of loaded cells provided evidence that liposomes and Tf-PEI mediated internalization of TT. As fluorescence labeling introduces negative charges into TT, the findings obtained by flow cyometry were confirmed by western blot analysis of cells loaded with unlabeled TT. Release of IFNg from mononuclear cells (MNCs) loaded with TT by liposomes or pA was clearly higher compared to passively loaded cells. In a human in vitro tumor model MNCs were pre-incubated with TT-xenogenized autologous lymphoblastoid cells and challenged with unmodified lymphoblastoid cells. In these cultures increased IFNg secretion was observed compared to MNCs derived from not xenogenized pre-stimulation cultures. Together, these data indicate the functional utility of the xenogenization strategy for the treatment of human neoplasias.

1431 ORAL

Anti-idiotype (anti-ID) vaccination plus intensive therapy (IT) and autologous stem cell transplantation (ASCT) for patients (PTS) with metastatic breast cancer (MBC)

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Purpose: A small proportion of pts with MBC achieve durable progression-free survival (PFS) with IT + ASCT. We are studying the effect of TriAb, an anti-Id vaccine and surrogate antigen for an epitope of human milk fat globule expressed on breast cancer cells with IT + ASCT in chemosensitive pts.

Methods: TriAb was given pre-ASCT weekly × 3 beginning 1 wk after the last cycle of conventional chemotherapy and monthly from day 7 post-ASCT & continued for 24 mo. or until progression. This design was to generate a primary immune response prior to collection of stem cells and IT. At the time of ASCT, 15 pts were in PR and 1 in CR. Treatment consisted of SC collection with a cyclophosphamide priming regimen followed by IT + ASCT with STAMP V.

Results: TriAb-related toxicity was minimal, with local injection site reactions and mild flu-like symptoms. No ASCT-related deaths occurred. High-titer IgG anti-anti-id (Ab3) responses were seen in all 16 pts at a median of 5 doses of vaccine. Three pts had disease progression at a median of 6 mo. post-ASCT; the others remain alive, without progression, at a median of 7 mo. post-ASCT.

Conclusion: Pre- and post-ASCT vaccination induces rapid Ab3 responses despite diminished-immunocompetency post-ASCT with minimal toxicity.

1432 ORAL

Humoral immune responses of cancer patients against 'Cancer – Testis' antigen NY-ESO-1: Correlation with clinical events

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Humoral and cellular immune responses against the 'Cancer – Testis' (CT) antigen NY-ESO-1 are frequently observed in patients (pts) with NY-ESO1-1 + tumors. This is in contrast to other known tumor-associated antigens (TAA) defined by antibody (Ab) or cytotoxic T cell (CTL) reactivity, i.e. MAGE Melan A, and tyrosinase, which induce immune responses in <10% of cancer pts. We showed previously, that high-titered NY-ESO-1 Ab and strong CTL against NY-ESO-1 can occur simultaneously. In healthy controls and pts with NY-ESO-1 – tumors, NY-ESO-1 Ab was not detected. In this study we assessed the NY-ESO-1 serum Ab response in pts with different NY-ESO-1 + tumors using Western blotting and ELISA. 10/12 patients had NY-ESO-1 serum Ab. All pts were followed for the development of NY-ESO-1 Ab titers under tumor treatment and clinical evolution. In 4 pts, an increase of NY-ESO-1 Ab titer was observed with progression of disease or extensive tumor necrosis. 1 pt showed a stable NY-ESO-1 Ab titer over 3 years along with gradual regression of a large tumor mass. In 5 pts, a decrease of