in paraffin-embedded sections was performed using standard techniques. P53 and bcl-2 were detected by primary monoclonal mouse anti-human antibodies in conjunction with red APAAP stain. Antigen-expression of 3 × 100 tumor cells per sample was evaluated by light-microscopy.

Results: In only 3 of 18 EOC (17%) and 1 of 6 ENC (17%) 10–90% of tumor cells were positively stained for p53. In contrast, 10–90% bcl-2-expressing tumor cells were found in 17 of 18 EOC (94%) and 5 of 6 ENC (83%). Samples with high expression of p53 (i. e. >50%) were also highly positive for bcl-2, whereas all bcl-2 negative specimens were found to carry wild-type p53.

Conclusion: In a substantial proportion of advanced EOC and ENC, bcl-2 appears to be activated by p53- independent pathways.

549 PUBLICATION

CA125 mimicry by SCFV-fragments of the monoclonal anti-idiotypic antibody ACA125 for immunotherapy of ovarian cancer

S. Reinartz, H. Schlebusch, P. Giffels, S. Köhler, D. Krebs, U. Wagner.
Dept. of Gynecology and Obstetrics, University Hospital of Bonn, Germany

Purpose: The $F(ab')_2$ fragment of the murine monoclonal anti-idiotypic antibody ACA125 mimicking the tumor associated antigen CA125 is used as a vaccine for the induction of an anti-tumoral immunity in patients with ovarian carcinoma. We tried to generate a single-chain fragment (ScFv) composed of ACA125 heavy and light chain variable domains.

Methods: Heavy and light chain genes of antibody producing mouse hybridoma cell line were separately amplified and assembled into a ScFv gene with linker DNA by PCR. The ScFv gene was ligated into the phagemid vector pCANTAB5E, which allows the production of both, phage displayed and soluble ScFv. Transformed *E. coli* TG1 cells were infected with M13K07 helper phage to yield recombinant phage, which display ScFv fragments as a g3p fusion protein on the surface of the filamentous phage M13. The *E. coli* non-supressor strain HB2151 was infected with an antigen-positive phage clone, previously screened by ELISA, to express soluble ScFv fragments.

Results: Functional soluble ScFv binding to the idiotypic antibody OC125 $F(ab')_2$ could be detected in the bacterial periplasm by Western blot and ELISA. The variable heavy and light chain genes of the ACA125 ScFv fragment were further sequenced and compared with known antibody sequences.

Conclusion: ScFv-Fragments of an anti-idiotypic antibody mimicking CA125 serves as the basis for a site-directed mutagenesis of the CDR-Regions in order to improve the immunological reactivity of the anti-idiotype vaccine.

550 PUBLICATION

The value of neoadjuvant chemotherapy (NACT) after surgical-pathologic staging (SPS) vs. failed debulking (FD) for inoperable ovarian cancer (IOC) and subsequent interventional laparotomy (ILAP)

D. Wallwiener, G.P. Breitbach, G. Bastert. *For the GTOC-Study Group; University of Heidelberg, Dept. of Obstetrics and Gynecology, Voss-Str. 9, 69115 Heidelberg, Germany*

Purpose: The role of a NACT for IOC (extensive peritoneal carcinomatosis and/or residuals (R) > 2 cm) after SPS and FD, respectively, was evaluated concerning remission, operability at ILAP und overall survival (OAS):

Methods: In a phase II multicenter study 64 patients with IOC after SPS or FD received a dose intensified NACT (Cisplatin 100 mg/m2/Treosulfan 5.000 mg/m2 i.v. + GCSF every 3 weeks, 3 cycles) with subsequent ILAP.

Results: To date, an ILAP was performed in 5 women with a FIGO-stage III (n = 24; 69%) und IV (n = 11; 31%) disease. A NACT was administered after SPS in 22 cases and after FD in 15. An R0 resection was possible in 53% of patients after SPS and in 27% after FD (p = 0.008). Remission (PR+CR) rates were 77% in the SPS and 76% in the FD group. The rate of PD und NC was comparable in the two groups.

Conclusion: Low toxicity of NACT (no WHO toxicity grade IV), good remission rates (76%) and operable findings at ILAP with the feasibility of optimal cytoreduction (R_0 43% and R_1 14%%) were achieved. The data also show a possible benefit in OAS in the SPS group. Final results are presently being evaluated.

551 PUBLICATION

High dose cyclophosphamide (C) and epirubicin (E) followed by cisplatin (P) and 5-fluoro-uracile (F) in patients with advanced epithelial ovarian adenocarcinoma (AEOC)

A.C. Braud, P. Cottu, J.M. Extra, M. Espié, C. Cuvier, M. Marty. *Dpt of Medical Oncology, Saint Louis Hospital, Paris 75010, France*

There are data supporting a dose-response relationship for alkylating agents in OC. High-dose P is neurotoxic but high-dose C has not been explored in combination chemotherapy with Anthracyclins in AEOC. We have studied since 1992 a densified regimen with 4 courses at 2 wks interval of C (1200 mg/m²) and E (75 mg/m²), followed by 4 courses at 3 wks interval of P (75 mg/m²) and F (1 g/m²/d × 3 by protracted infusion). 29 stage IIIC–IV pts, with a median age of 53 y (range 35–73) were included. Other eligibility criteria were PS: 0–2, no cardiac nor renal contra indication to E or P. 55% of pts had >2 cm residual implants after primary surgery. CE courses were administered whatever the WBC count, without hematopoietic growth factor, but were delayed if febrile neutropenia. Toxicities differed in the 2 parts of treatment. Grade 4 neutropenia was more prominent after CE (70%), but neutropenic fever was unfrequent (14%). Grade 2–3 anemia and Grade 3 emesis were more frequent after FP (39 and 46% respectively). Clinical CR was achieved in 72% of pts. Among 19 pts with objective response assessed by second look laparotomy, 9 obtained pathological CR (47%). After a median follow up of 25 months, 17 pts have progressed and 12 died. Median progression free and overall survival were 13 and 28 months, respectively. These results are in the same range than those of C-P regimens, despite a lesser cumulative dose of P.

552 PUBLICATION

Stimulation of cytotoxicity and cytokine production in tumor – Associated macrophages from ovarian, breast and lung cancer patients

E. Zavadova, E. Pedram, E. Hauptmann, L. Desser, M. Micksche. *Institute for Tumor Biology/Cancer Research, University of Vienna, Austria*

Purpose: The role of tumor-associated macrophages (TAM) as potential effector cells for eradicating malignant cells is not vet entirely clarified. In the present study TAM were phenotypically and functionally characterized. The second part of our investigation was the activation of TAM by cytokines rhGM-CSF, rhIFN-γ or with the polyenzyme preparation Wobe-Mugos used in the treatment of cancer patients with malignant effusions.

Methods: TAM were isolated from malignant effusions of breast, ovarian and lung cancer patients by gradient separation and characterized, by the following parameters: surface epitopes (moAb 27E10, 25F9), respiratory burst activity, cytotoxicity and TGF-β production measured in culture supernatants by ELISA/RIA. Additionally mRNA of TGF-β was detected in TAM by in situ hybridization.

Results: Incubation of TAM with GM-CSF, IFN-γ and polyenzyme preparation Wobe-Mugos resulted in an augmentation of cytotoxicity. In contrast, GM-CSF, IFN-γ and Wobe-Mugos reduced the production of TGF-β by TAM, as verified by ELISA assay as well as by in situ hybridization. TGF-β is known as an immunosuppressive molecule.

Conclusion: Our studies show that the cytotoxic capacity of TAM obtained from malignant effusions of cancer patients can be increased by GM-CSF, IFN-γ and Wobe-Mugos. TGF-β release was reduced. Whether this observation is of therapeutical relevance has to be determined by further studies.

553 PUBLICATION

The integration of consolidation whole abdominal irradiation in the multi-modality management of advanced ovarian carcinoma: Long-term results

A. Kuten[1], H. Goldberg[1], M. Steiner[2], E. Bar-Shefi[1], D. Beck[1], R. Rubinov[2]. *Departments of Oncology, [1]Rambam Medical Center; [2]Lin Medical Center; Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel*

57 patients (pts) with advanced ovarian carcinoma were treated by primary cytoreductive surgery followed by 6–11 courses of cisplatin (50 mg/m²) and adriamycin (50 mg/m²), second look laparotomy (SLL) (46 pts) and consolidation whole abdominal irradiation ± pelvic boost. Only 42/57 pts (74%) tolerated radiotherapy (RT) and were able to receive the full planned whole abdominal RT dose of 30 Gy. The median follow-up time is 39 months (range, 12–197 months). The following 5 and 10 year actuarial

survival figures were obtained from pts who were able to complete the full course: all 42 pts· 44%, 26%; St II: 40%, 40%; St III–IV: 45%, 24%. Gr I–II: 53%, 41%; Gr III: 37%, 11%. Residual tumor at primary surgery: none: 75%, 56%; < 2 cm: 49%, 31%; > 2 cm: 26%, 7%. Negative SLL/microscopic disease at SLL: 63%, 31%. Macroscopic disease at SLL: 19%, 10%. The survival probability of 11 pts who were irradiated following clinical CR achieved by chemotherapy (CT) without SLL was 46% and 34% at 5 and 10 years, respectively.

Conclusions: 1) Residual tumor at primary cytoreductive surgery and SLL outcome are both prognosticators of survival. 2) In view of the promising results, the integration of consolidation RT as part of the multi-modality management of advanced ovarian carcinoma, especially in complete responders to CT, should be investigated in Phase III studies.

554 PUBLICATION

Use DNA flow cytometry (DNA FCM) in diagnosis and prognosis of serous boderline tumors and high grade ovarian cancer

I.V. Panitchenko[1], V.N. Bogatyrev[2], K.I. Jordania[1], M.A. Shibanov[2].
[1]Department of Gynecology, Laboratories of Clinical Cytology; [2]Pathology, N.N. Blokhin Cancer Research Center, Moscow, Russia

Purpose: The aim of this study was to assess the possible use of DNA FCM in the daily clinical practice for differential diagnosis and prognosis of serous papillary boderline tumors (SPBT) and high grade serous adenocarcinoma (HGSA) of ovary.

Methods: DNA FCM (FACScan, Becton Dikinson; software Multicycle, Phoenix Flow Systems, USA) was performed in 9 cases of SPBT, 23 HGSA cases.

Results: The proliferative rate (18.9 ± 1.2%) of aneuploid SPBT was higher than diploid ones (7.1 ± 2.2%) and same parameters of HGSA (16.6 ± 2.4%) as well as higher and same proportion of S phase cells (7.2 ± 0.6% versus 3.5 ± 0.3% and 8.1 ± 1.3%). In these cases the only morphological differentiation between aneuploid SPBT and HGSA was very difficult. Therefore, cytopathologist, using the data of DNA FCM, may think of HGSA instead of SPBT. It was found that aneuploid HGSA occurred in 87.5% and diploid ones in 12.5% cases. The analysis of distant results has shown that patients with aneuploid tumors have higher chance of progressive disease.

Conclusion: Thus, our data has demonstrated the obvious value of DNA FCM for improving accuracy in diagnosis and prognosis of SPBT and HGSA.

555 PUBLICATION

Palliative complex treatment of the patients with ovarian cancer by using of radiation component

L. Vorobyova, A. Vinnitskaya, Yu. Dotsenko, G. Yevtushenko, V. Svintsitsky, L. Gulak, A. Turchak. *Department of Oncogynaecology, Institute of Oncology and Radiology, Kiev, Ukraine*

Purpose: To detect the role and the place of radiation therapy in complex treatment of the ovarian cancer patients and to improve the results of palliative treatment.

Methods: 86 patients with II–III stages of ovarian cancer (T2–3 No-1 Mo) at the age between 17 and 69 were treated. Telegammatherapy has been conducted using "Rocus" apparatus from two opposite fields 16 × 16 cm in static regimen and 6 × 16 cm – in a mobile one. Single site dose was 2–2.2 Gy, total – 35–40 Gy. Radiation component has been used in complex therapy of the patients with nonascitic forms of ovarian cancer with operation at a full volume (panhysterectomy with omentectomy), in residual tumors in a small pelvis, but without metastases to the organs.

Results: Radiation treatment has been conducted on the background of intraperitoneal and intravenous PCT (platidiam, adriamycin, endoxan, vincristine). Use of radiation component in complex therapy gave more prolonged remission as compared to the combined method (operation + PCT): 42.6 ± 3.8 months and 34.8 ± 3.6 months, respectively. 5-year survival in complex treatment of the patients with II–III stages of ovarian cancer was 62.4% as compared to 45.7% in the combined treatment.

Conclusion: Inclusion of the radiation component under the above conditions to postoperative therapy of the ovarian cancer patients promotes prolongation of the clinical remission and survival.

Cancer in children

556 ORAL

Metastatic osteogenic sarcoma (OS) at diagnosis. Study of 73 cases from the french society of pediatric oncology (SFOP) between 1980 and 1990

H. Pacquement, C. Kalifa, C. Fagnou, M.C. Demaille, M. Brunat-Mentigny, E. Sariban, Y. Perel, J.M. Zucker. *Institut Curie for the SFOP, Paris, France*

In order to assess the prognostic value of a good histological response (GR) in metastatic OS at diagnosis, 73 patients (pts) were retrospectively studied.

Patients: 36 boys, 37 girls, 3 to 19 years (med 13) of age. The site of the primitive tumor (PT) was distal femur (40), proximal humerus (12), proximal tibia (11), other (10). 66 pts had pulmonary metastases (8 only one metastasis, 41 multiples pulmonary sites, 14 associated with bone, 3 combined sites). 5 pts had only bone metastasis. 2 pts had combined sites (liver, regional node).

Treatment (tt): All pts received chemotherapy (CT) with (48) or without (25) Cisplatinum (CDDP); pts without CDDP received methotrexate and adriamycin. Local tt was possible in 60 pts: radiotherapy only in 18 pts, surgery in 52 pts. The histologic response is evaluable in 41 pts: 21 GR, 20 poor response (PR). In 30 pts, lung metastases were resected. 36 pts did not have thoracotomy.

Results: 23/73 pts went CR for PT and metastases; 15/23 pts relapsed. 43 pts died 2 months (mo) to 63 mo after diagnosis (med 15 mo), 17 are lost for follow-up with tumor. 13 pts are alive in CR with a median follow-up of 7 y; none of the alive pts had bone metastasis. Overall survival for all pts is 15%, GR 50%, PR 5%.

Conclusions: 1) this study confirms the poor prognosis value of bone metastasis at diagnosis. 2) the survival depends on the achievement of CR and histological GR (12 alive pts/21 GR, 1 alive/20 PR). 3) 4/8 pts with only 1 pulmonary metastasis are alive; the 4 dead had no surgery for their PT. 4) surgery is necessary for the PT.

557 ORAL

Adaptation of treatment to clinical presentation in stage 4S neuroblastoma. Results of the SFOP NBL90 study

J. Michon[1], O. Hartmann[2], C. Bergeron[2], P. Chastagner[2], H. Rubie[2], C. Coze[2], J.M. Zucker[2], D. Plantaz[2], M.C. Baranzelli[2]. *[1]Departement de Pédiatrie Oncologique, Institut Curie, Paris, [2]Neuroblastoma Group of the SFOP, France*

Purpose: Clinical presentation of patients (pts) with 4S neuroblastoma (NB) is heterogeneous, and standardization of treatment (Tt) is difficult. We established a Tt scheme adapted to severity of respiratory symptoms and to evolution of metastases.

Methods and Patients: After diagnosis and staging, pts with respiratory distress received 1.5 Grays/day (d) for 3 d by external beam radiotherapy (RT) on the liver. Chemotherapy (CT) was associated, or preceded RT, if necessary and made of Vincristine 0.05 mg/Kg/d D1 and Cyclophosphamide 5 mg/Kg/d D1 to D5 (CO) for 6 courses; in case of progression, 2° line CT was given, consisting of 2 courses of VP16-Carboplatinum. Pts with no respiratory distress were monitored as outpatients and were planed to receive CO and/or RT in case of disease progression. In all cases, the primary was surgically removed 4 to 6 months after diagnosis. 65 pts diagnosed between 01.90 and 03.96 (36 M and 29 F) are evaluable. Age at diagnosis was 0 to 196 d (med 49 d). Primary was located in the abdomen in 56, in the mediastinum in 3, in the neck in 1 and not found in 5 pts. Metastatic sites were: subcutaneous tissue (12), liver (59), bone marrow (16).

Results: The 6 pts without liver disease are alive – 23/59 pts with liver disease received no CT during the first 2 months after diagnosis: 20 had spontaneous remission, and 3 were treated for progression; all are alive. Among the 36 pts who received early treatment, 2 had liver RT only, 12 CO only, and 22 RT ± CO ± 2° line CT. Only 3/16 pts treated by CO first had a response. 2° line CT was needed in 8/11 receiving CO + RT. 9 responses were observed in 11 patients receiving VP16-Carboplatinum. 14 events and 10 deaths were observed. Overall survival of the cohort is 84% ± 7 with a median follow-up of 65 months.

Conclusions: VP16-Carboplatinum should be used for pts with 4S NB, who need a Tt.