by sc IL-2 for 10 days (days 6–15) and then by 15 day rest (days 16–30). IL-2 daily dose was escalated, starting from 250 MIU in subsequent groups of three pts, according to the Fibonacci's schedule, every two cycles.

Results: 15 pts (8 colon, 1 lung, 1 pancreas, 3 renal and 1 prostate carcinoma and 1 soft tissue sarcoma) entered the study. Thirty cycles were completed and a maximal IL-2 dose of 2,500 MIU was achieved without significant side-effects. Granulocyte, monocyte, dendritic (CD34+, CD14+, CD80+, CD11c+, HLA-I+, HLA-Dr+) cell, and NK (CD3−, CD56+, CD16+, CD11b+) increase was observed in all pts after treatment. GM-CSF/IL-2 also increased the CD4/CD8 ratio in 13 pts who previously presented an inverted CD4/CD8 ratio.

Conclusions: these preliminary results suggest that GM-CSF and IL-2 combination is not toxic and its biological activity in cancer pts might be useful to support anticancer active TAA-specific immunotherapy by increasing APC activity and T cell immune-response. Supported by a grant from MURST (ex-40%).

1451 PUBLICATION

Long term follow up of 50 patients with metastatic renal cell carcinoma treated with high dose i.v. interleukin. 2

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From 7/89 to 10/93 50 patients (pts) with metastatic renal cell carcinoma (RCC) were treated with rIL-2 at the close of 18×10^6 I.U./m^2/day continuos infusion for 5 days, 2 days rest and 5 additional days every 3 weeks for 2 cycles; in pts with response or stable disease 4 additional 5 days cycles were administered. The pts characteristics were: 35 M, 15 F, median age 59 (32–76) years; median PS 1 (0–2); the metastatic sites were only lung in 20 pts; 20 pts were pretreated. All pts were considered evaluable for toxicity: 38% of the pts had at least 1 G 3–4 episode, 2 treatment related deaths (infection after polmonary toxicity) occurred. A total number of 268 cycles was administred and all pts who could receive at least 1 cycle (45) were considered evaluable fo response. We observed 4 CR, 5 PR, 16 NC, with similar duration (8 months); the median (range) survival (months) of the CR is 86 (7–112), PR 24 (12–76), NC 28 (7–87), PD 8 (1–44); 2 CR pts are still disease free after metastasectomy since they had only one site of relapse (thorax). Out of the prognostic factors considered (sites of disease, total received dose, lymphocitosis and eosinophilia, WHO-PS, age) only the P.S. was correlated with the survival. Lymphocitosis (100% increase of lymphocites count after the first cycle) occurred in 100% of CR, 70% of PR, 100% of NC, 70% of PD and thereafter, in our experience, it couldn't be considered a "biological marker" of response. In conclusion: this long term follow up shows that a small percentage of RCC pts treated with high dose i.v. rIL-2 can have a significative increase of survival and some of them after surgery may be considered cured.

1452 PUBLICATION

Fas ligand (CD95L) induction in human lymphocytes by the apoptosis-inducing mistletoe lectins

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Purpose: Fas ligand (FasL) triggers apoptosis in Fas receptor (Apo-1)-positive target cells. We investigated the expression of FasL, Fas and TNF receptor 1 (TNF-R1) on cultured human lymphocytes and leukemic T and B cells.

Methods: Cell surface molecules were measured by flow cytometry in lymphocytes from 6 healthy individuals, from 4 patients with chronic lymphocytic T or B cell leukaemia, and leukemic Molt-4 cells incubated for 72 h with the apoptosis-inducing mistletoe lectins (ML I and ML III) at 10 ng/ml.

Results: ML significantly induced apoptosis in a fraction of lymphocytes, while in the surviving $CD4^+$ T helper cells, $CD8^+$ cells and $CD19^+$ B cells, FasL and TNF-R1 was upregulated, while the Fas molecule decreased. In contrast, FasL was not induced in leukemic cells. This may reflect distinct 'activation' of the surviving cells, which did not result in a proliferation response as measured by the expression of CD25 and CD71, or nuclear Ki-67 antigens. Surprisingly, the apoptotic cells showed increased level of intracellular IL-4, indicating that apoptosis and tolerance are linked through the production of anti-inflammatory cytokines to prevent deleterious immune responses.

Conclusions: Apart from a direct induction of apoptosis in response to an inhibition of protein synthesis by the enzymic ML A chain, ML treatment may indirectly induce apoptosis in Fas^+ tumour cells through activated $FasL^+$ lymphocytes. As ML-rich whole plant extracts from *Viscum album* L. are applied as an adjuvant in complementary cancer therapy, an implicated clinical relevance of their FasL-inducing properties has to examined carefully.

1453 PUBLICATION

Alteration of expression in c-erbB2, bax, p53, bcl-2, JNK, p21 and PKC/c-myc induces PCD in breast carcinoma after adm. of hexadecyl-PC, antiHER2-mAbs & vinorelbine conjugates

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Purpose: HER/neu gene is overexpressed in 30% of human breast cancers, and it is associated with p53 abnormalities, aneuploidy, intrinsic multidrug resistance due to inhibition of apoptosis, augmented DNA repair mechanisms, DNA synthesis, cell growth, mitotic rate, tumourigenicity and metastatic potentiality leading to poor prognosis.

In this study, we aim to find if there is therapeutic utility of anti HER2-IgG bearing fusogenic immunoliposomes consisting of PKC inhibitor-hexadecylPC with encapsulated anti-mitotic vinorelbine-tartrate against advanced breast carcinoma, which exhibits overexpression of tumour suppressor gene p53, and protooncogene HER-2/neu, due to mutations.

Methods: A patient with axillary node metastasis, secondary to a breast primary has been treated with mastectomy. From this specimen, tissue was treated with collagenase and tumour cells were isolated. Paraffin-embedded formalin fixed tissue was analysed by IHC with relevant antibodies for ER, PgR, HER-2/neu, bax, p21, p53 and bcl-2. JNKmRNA and PKCmRNA were measured by Northern blot. Apoptosis was assayed by transmission electron microscopy. Tumour cells were analysed before and after treatment with vinorelbine encapsulated in antiHER2-IgG bearing fusogenic liposomes consisting of hexadecyl-PC.

Results: The breast carcinoma was identified as hormone independent. After treatment, immunochemical analysis has exhibited upregulation of p21, bax, c-myc and downregulation of c-erbB2, bcl-2 compared to measurements before treatment. Expression of p53 remained enhanced due to mutations in the middle region of exon 6 (AA 212–217) before and after treatment. Post-treatment measurement with Northern blot has exhibited enhanced expression of JNK, and reduced expression of PKC compared to pre-treatment assay measures. TEM has exhibited irreversible D2 stage of apoptotic signs with formation of apoptotic bodies, which are phagocytosed by adjacent tumour cells implying a by-stander effect.

Conclusion: We have achieved to eradicate chemoresistant human breast carcinoma cells by apoptosis mediated by the kinase activity of JNK, circumventing mtp53.

1454 PUBLICATION

Lyophilized whole human melanoma cells enhanced suppressive action of PBMC toward survival of the corresponding malignant cell line in vitro

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Purpose was to determine: a) Does the peripheral blood mononuclear cells (PBMC) six-days-preincubation in nutrient medium with human AB serum with lyophilized human melanoma BG or Fem-x cells affect their antiproliferative action towards the corresponding malignant cell line in vitro and b) Does the PBMC six days preincubation with lyophilized normal PBMC, obtained from healthy volunteer (as a source of allogenous, but not of tumor antigens), affect their suppressive action on the survival of both melanoma BG and Fem-x cell lines in vitro.

Lyophilization of malignant cells, as well as of normal PBMC obtained from healthy volunteer, was done by freezing the suspension of whole cells in nutrient medium with normal human AB serum at −80°C. The frost suspension was dehydrated in high vacuum, in lyophilizer. Determination of the antiproliferative action of the untreated (naive), or of six days stimulated PBMC on malignant cells, was also done by MTT test.

Results showed that six days stimulation of PBMC with lyophilized whole BG cells enhanced their suppressive action towards the survival of BG cells in 17 from 19 investigated cases. Six days stimulation of normal PBMC with lyophilized Fem-x cells enhanced their supression of Fem-x cell survival in 8

from 13 invetigated cases. The extent of this enhancement varied between individuals and was an invert function of the antiproliferative action of naive PBMC. On the contrary, six days preincubation of normal PBMC with the lyophilized healthy PBMC inhibited PBMC their suppressive action towards the survival of both malignant cell lines in vitro in 8 out of 9 investigated individuals.

1455 PUBLICATION

Lyophilized whole melanoma cells and irradiated whole melanoma cells stimulate PBMC suppressive action toward melanoma cells survival

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The most attractive biological approach to eradicate the disseminated neoplasm is the induction of an antitumor immune response. The goal of this work was to compare the potency of stimulating irradiated BG cells and of lyophilized whole BG cells to enhance the peripheral blood mononuclear cells (PBMC) suppressive action on the survival of melanoma BG cells, and to check if there are any specificity in the induction of the antitumor immune response regarding lyophilized melanoma cell lines used for PBMC stimulation, in vitro.

Lyophilization of malignant cells, was done by freezing the suspension of whole cells in nutrient medium (with 10% AB+ human serum) at −80°C. The frost suspension was dehydrated in high vacuum, in lyophilizer. Lyophilized cells did not exclude trypan blue, and were with markedly stained membrane and nuclei. Irradiation of BG cells was done with 30 Gy by x rays (X-6 MV, CLINAC 2100C, Varian). Irradiated BG cells, although exclude trypan blue, were reproductive dead, e.i. they did not formed colonies in vitro. Determination of the antiproliferative action of the untreated (naive), or of six days stimulated PBMC on malignant cells, was done by MTT test.

Results showed that six days stimulation with lyophilized melanoma BG cells enhanced the suppressive action of PBMC towards the survival of the BG cells in five from six investigated cases, in comparison with the action of naive PBMC. Six days preincubation of normal PBMC with the irradiated BG cells led to the increase in their suppression of BG cells survival in two from six investigated cases. Six days preincubation of PBMC with lyophilized BG, or Fem-x cells induced the enhancement of their antiproliferative action toward both investigated cancer cell lines; the extent of this enhancement was an invert function of the antiproliferative action of naive PBMC and was not dependent on the specificity of the melanoma cell lines used.

1456 PUBLICATION

Interferon alpha and its various functional facets in comparison with other cytokines (AC) in an in vitro setting

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Purpose: To study the efficacy of interferon alpha (IFNα) on human tumour cell lines.

Methods: To use colorimetric and biochemical techniques to study the biological activities of IFNα.

Results: 1. Whilst both IFNα and AC upregulated MHC class I antigens, class II antigens were only induced by AC. These results were also demonstrable for intracellular cell adhesion molecule (ICAM-1). 2. Both IFNα and AC increased the killing activity of IL-2-activated mononuclear cells (LAK) cells by as much as 15% percent. In addition, pre-treatment of tumour target cells with IFNα increased their susceptibility to killing. 3. IFNα and AC showed direct cytotoxic effects on some tumour cell lines like Wil (a bladder line). 4. Combination of IFNα and cisplatin showed additive suppressive effects on tumour lines.

The findings of this investigation demonstrated the capacity of IFNα to increase the visibility of tumour cells to the immune system by increasing the expression of MHC class I antigens. The data also demonstrated that IFNα acted at other levels contributing to its overall clinical efficacy.

Supportive care & quality of life

1457 ORAL

Cross-language validation of the Functional Assessment of Cancer Therapy-Anemia (FACT-An) questionnaire

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Purpose: Quality-of-life (QOL) data from a randomized, placebo-controlled clinical trial to assess the effect of epoetin alfa on cancer-related anemia were analyzed to evaluate the measurement characteristics of 6 language versions of the Functional Assessment of Cancer Therapy-Anemia (FACT-An) questionnaire. Classical test theory and modern item response theory were used to test the validity of pooling data across languages to support the investigation of an epoetin alfa effect on QOL.

Methods: The FACT-An includes the FACT-General (G) and the FACT-An Fatigue scales, determined a priori as primary QOL endpoints. FACT-An data in 6 languages (Dutch, French, German, Italian, Portuguese, and English) were available from 317 patients enrolled in the study. Traditional reliability and Rasch rating scale analyses were applied, testing for items performing differently across languages.

Results: Internal consistency was high across all languages for both FACT-An primary endpoints for the study: FACT-G Total score (.83–.91) and Fatigue Subscale (.89–.92). Pairwise language comparisons of item difficulty calibrations revealed only 21 of 200 (10.5%) cases of differential item functioning for the FACT-G and Fatigue items comprising these 2 Rasch measures, enabling the pooling of data for these scales in this multinational study. Other FACT-An scales demonstrated similar consistency.

Conclusion: These results support pooling FACT-An data across languages and demonstrate the value of using the FACT-An as a QOL assessment tool in international clinical oncology trials. These findings substantiate the overall QOL treatment effect observed in this multinational trial.

1458 ORAL

Influenza vaccination in patients with cancer

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Purpose: Influenza is the meet dangerous seasonal infection of our century. Especially patients with compromised immune system are at risk for severe influenza-related complications. Therefore, vaccination is strongly recommended for these patients. However, in cancer patients, outcome of influenza immunisation is often doubted to be sufficient due to anticancer therapy or cancer activity. The aim of this study was to prove if antiviral protection can be achieved by vaccination in patients with malignancies.

Methods: 56 patients with solid tumours or hematologic neoplasms (28 of which underwent chemotherapy) and 45 healthy individuals were immunised with trivalent influenza vaccine (A/Sydney/5/97 (H3N2); A/Beijing/262/95 (H1N1); B/Beijing/184/93). Peripheral blood was sampled before and four weeks after vaccination, and titers were determined by hemagglutination inhibition tests.

Results: After vaccination, 55% of all patients and 87% of the healthy subjects developed a protective titer ($\geq$1:40) against at least one influenza subtype. Interestingly, patients under chemotherapy showed no significantly different postvaccinational titer compared to that of the unseated patient group ($p = 0.6$). However, among patients with solid tumours ($n = 32$), immune responses were higher than in patients suffering from lymphatic neoplasms ($n = 18$, $p < 0.05$). No severe side effects were observed.

Conclusion: The majority of cancer patients developed an appropriate influenza immune response irrespective of concurrent anticancer treatment. These results strongly recommend influenza vaccination for patients with malignancies.