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