surgery in 12 with local control in 10 of 12. The 4 patients treated by radiation alone have local control (4/4); 3 of these were recurrent following prior surgery. The two patients who failed locally after surgery and post-operative radiation are alive without evidence of their DFSP at >2 years following salvage surgery.

This limited experience demonstrates that radiation is an effective therapeutic modality against DFSP and can be recommended for those patients who are inoperable for medical or technical reasons or for whom the functional cosmetic price would be unacceptable to the patient.

219 POSTER

SECOND PRIMARY TUMOURS IN MELANOMA PATIENTS

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Many authors report a high incidence of second primary tumours in patients affected by cutaneous malignant melanoma. We treated 369 patients (pts) suffering from malignant melanoma; they were submitted to surgical treatment related to the stage of disease; 196 (53.1%) of these patients had undergone systemic or locoregional chemotherapy; 41 pts (11.1%) had systemic immunotherapy with α-interferon. The mean follow-up was 39.7 months, median 30 months. Among all patients, 27 (7.3%) had a second primary neoplasm, diagnosed either previously (16 pts, 59.3%) or after (11 pts, 40.7%) melanoma diagnosis. For the entire group, there were five pts with breast cancer, four with head and neck cancer (1 tongue, 1 larynx, 2 thyroid), five with gynecologic tumours (3 with uterine and 2 with ovarian cancer), three with skin tumors (2 basal cells tumour and 1 further melanoma), two myeloproliferative tumours (1 Hodgkin lymphoma and 1 thrombocytosis), one with soft tissue sarcoma, one with lung cancer, four with gastrointestinal cancers (2 colorectal and 2 gastric cancer), two with urinary tract (1 kidney and 1 bladder cancer). The differential analysis of gender and age showed a trend for increasing incidence of the second cancer in females than males ($P = 0.23$, ns) and found that risk for the second tumour was higher in older ages ($P = 0.02$). Site, Breslow thickness, and therapy of primary melanoma were not predictive for a second cancer. Moreover, we found that breast and uterine cancer (hormone related cancer) was common in the group of neoplasms preceding melanoma. The incidence of second primary warrants a more careful follow-up in melanoma patients and their relatives.

220a POSTER

IMPLICATION OF THE GLUTATHIONE ON B16 MELANOMA CELLS SENSITIVITY TO CYCLOPHOSPHAMIDE

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Depletion of glutathione (GSH) has been proposed as a useful antitumour approach, increasing cellular sensitivity to other anticancer. GSH is a ubiquitous tripeptyde thiol that has a critical role in protection of cells against the cytotoxic effects of a wide variety of drugs, such as activated cyclophosphamide (CY). It has been demonstrated that GSH can protect against the DNA cross-linking effect of this bifunctional alkylating agent, with consequent depletion of intracellular GSH levels. The depletion of GSH content following exposure to activated CY has been attributable to intracellularly released acrolein. Recently, we have shown in studies *in vivo*, that B16F10 melanoma cells are sensitive to CY treatment. Thus, high-dose CY therapy (300 mg/kg) significantly ($P < 0.001$) decreased the number and growth rate of B16 melanoma liver metastases, reducing the hepatic volume infiltrated by metastatic tissue and, therefore, increasing significantly ($P < 0.001$) the survival time of tumor-bearing treated mice in comparison to the control group. We observed that in the set of mice treated with CY the metastases became amelanotic whereas in the control group remain strongly pigmented. Because GSH is also involved in cell proliferation and in the melanin synthesis, in the present study we assessed, *in vitro*, the effect of acrolein on GSH levels of B16F10 melanoma cells. We observed that acrulein (10 μM) significantly ($P < 0.005$) decreases the GSH levels and cell proliferation of these tumour cells. It is concluded that B16 melanoma cells are sensitive to CY therapy, decreasing the proliferation and melanogenesis of these tumour cells, and that depletion of intracellular GSH levels could be one of the possible mechanisms implicated (supported by UPV/EHU grant number 075.327 E117/94.)

220b POSTER

INTRAVENOUS HIGH DOSE THYMOPENTIN IN METASTATIC MELANOMA PATIENTS: EVALUATION OF TOXICITY AND CLINICAL EFFICACY

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The study concerns a group of eight cases, firstly treated with 3 different modalities of dosing: Group A: 3 patients were treated with systemic Thymopentin (TP5) 1 gr/three times a week i.v. for 5 weeks; Group B: 3 patients received systemic TP5 1 gr/daily i.v. for 5 weeks; Group C: 2 patients had systemic TP5 2 gr/daily i.v. for 5 weeks. The clinical evaluation criteria included an immediate control at the end of dosing and a second check four weeks after ending therapy. The therapy was well tolerated by all the patients and no impact was produced on the daily activities or qualify of life of treated cases. As far as concerns cutaneous metastases we obtained four Partial Responses and four Stable Disease. The duration of response ranged from 1 to 8 months (mean 4). Actually 6 patients are alive with disease and 2 patients died of disease.

Clinical and pathological data are indicating that i.v. high dose TP5 is an agent able to produce consistent biological and immunological effects in advanced melanoma patients, especially if cutaneous or subcutaneous metastases are considered.

221 POSTER

ACTIVE SPECIFIC IMMUNOTHERAPY (ASI) IN PATIENTS WITH METASTATIC MELANOMA (MM)

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Background: ASI has some promise in the treatment of patients (pts) with MM. Several clinical studies have demonstrated both humoral and cellular responses against melanoma antigens *in vitro* and an influx of tumor infiltrating lymphocytes in regressing metastatic lesions in MM pts. In general, vaccination with whole cell vaccines, either autologous or allogenic results in generation of melanoma specific delayed type hypersensitivity (DTH) reactivity *in vivo*.

Objectives: The purpose of our study was to reach maximal DTH response in individual MM pts and to evaluate whether the magnitude of these responses correlated with (progression-free) survival.

Methods: 44 MM pts were treated with autologous cell vaccines (2–16x, the first 2 admixed with BCG). Twenty-two pts had no evidence of disease after metastatectomy (NED group), neither clinically nor pathologically. The other 22 pts (ED group) had either incomplete resection of MM (n = 9) or still clinical evidence of MM (n = 13). Vaccines were prepared from material obtained after surgery according to a standardized procedure.

Results: The majority of pts in the ED-group (median survival 7 months) did not show an important DTH response. Peripheral blood lymphocytes (PBLs) showed strongly decreased responses to mitogens and PPD, which did not change after vaccination. In contrast, the majority of pts in the NED group (median survival 16^+ months) showed a high DTH response and DTH reactivity correlated with survival. PBL responsiveness to PPD was strongly enhanced after vaccination.

Conclusion: In particular, in NED MM pts improvement of vaccine preparation by maneuvers, which enhance DTH response should be further explored.

222 POSTER

INDUCTION OF SPECIFIC T-CELL RESPONSES *IN VITRO* AND *IN VIVO* WITH MELANOMA ASSOCIATED PEPTIDES

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Specific cytotoxic T-lymphocyte (CTL) activity against autologous melanoma cells is often generated by mixed tumor lymphocyte cultures (MLTC) *in vitro*. Targets for CTL responses are peptides of 9–10 aminoacids presented by MHC class I molecules. From HLA-A2+ melanoma systems extensively studied in our laboratory several antigenic peptides were recently characterized by molecular cloning and surface stripping of HLA class I bound peptides. All antigens identified are differentiation antigens of the melanocyte lineage (tyrosinase, gp100, Melan A). Their potential role for tumor rejection is currently unknown. To evaluate the toxicity and immunologic effects of intradermal injection of these peptides, a pilot study was conducted. Three patients (HLA A2+) with metastatic melanoma, who failed on conventional treatment protocols, were injected weekly with 100 μg of peptides derived from Melan A (4 variants), tyrosinase (3 variants), gp100