performed in 42 patients (25 men and 17 female, aged 28 - 81 years) with stage III and IV MM. Results were correlated with other clinical tests.

Results: Tyrosinase RT-PCR was positive in 8/29 patients with stage III and in only 2/13 patients with stage IV MM. In 5/8 patients with positive tyrosinase systemic metastases already developed despite short follow-up (0-9 months). In a group of 21 patients with negative tyrosinase only 3 developed systemic metastases. S-100 protein was normal (<0.01 g/L) in 25 and elevated in 4 patients with stage III MM. Systemic metastases developed in 5/25 with normal and in 2/4 with elevated S-100 protein. There was a positive tyrosinase reaction in 3/5 patients with normal S-100 protein who developed systemic metastases. With a combination of tyrosinase RT-PCR and S-100 protein we were able to predict systemic metastases in 5/7 patients.

Conclusions: Positive tyrosinase in peripheral venous blood is a better predictor of systemic metastases than serum S-100 protein. However, since there are cases with negative tyrosinase and elevated S-100 protein, we recommend the combination of both tests. Moreover, with longer follow-up we can expect these results to became even better.

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Evaluation of the potential immunomodulating benefit by the application of retinoic acid in chemoImmunotherapy of metastatic melanoma

G. Konjevic, V. Jovic, M. Mandic, S. Radulovic, S. Jelic, I. Spuzic. *Institute for Oncology and Radiology of Serbia, Belgrade, Yugoslavia*

Purpose: Considering contradictory reports regarding the potential beneficial therapeutic effect of 13-cis-retinoic acid (RA) in combined chemoimmunotherapy trials for metastatic melanoma (MM), the aim of this study was to perform detailed immunological evaluation of patients undergoing different chemoimmunotherapeutic regimens with or without RA.

Methods: 35 MM patients were treated with DTIC, 800 mg/m2/day and interferon alpha-2a (IFN), 5x106 IU/m2/day s.c., during 5 days (group A) and 35 MM patients received the same regimen, supplemented with RA, 60 mg/day, during 10 days (group B), and compared to 39 healthy controls. Peripheral blood lymphocytes (PBL) NK cell activity, PHA-induced proliferation (LTT), CD4+ and CD8+ T cell and NK cell subsets wereanalysed on day 1, 6 and 28 of the first three therapy cycles. The same parameters as well as the dynamics of IRF-1 transcription were evaluated on in vitro treated PBL with IFN, RA and IFN+RA.

Results: Predictive in vitro treatments of PBL showed a significant synergy in the expression of IRF-1 mRNA, and all the other evaluated parameters in combined IFN + RA treatments. However, immunological monitoring showed only significant increase in NK cell activity on the day 6 of the 1st therapy cycle in both groups, and an increase in CD4+ T cells on day 6 of the 1st cycle in group A. In the expression of CD69 on CD56+ PBL and CD38 on CD8+ T cells there was a repeating pattern of increase on day 6 of each therapy cycle in both groups, contrary to the gradual increase in HLA-DR expression on CD3+ T cells in group A, and an early decrease in group B.

Conclusion: The obtained results suggest that contrary to the observed in vitro synergism between IFN and RA, there was no immunopotentiating, nor therapeutic benefit in the regimen that included RA.

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Isolated limb perfusion with fotemustine after chemosensitization with dacarbazine in melanoma

J.L. Pontes[1], M. Lopes[2], M. Ribeiro[1], J.L. Guimarães[1], J. Videira[1], C. Azevedo[2], G. Santos[1]. *[1] Instituto Português de Oncologia, Surgical Oncology I, Porto, Portugal; [2] Instituto Português de oncologia, Medical Oncology III, Porto, Portugal*

Introduction: Isolated Limb Perfusion (ILP) with Melphalan continues to be the standard treatment for localised recurrent melanoma of the limbs (Stages III - M. D. Andersen). In terms of local and regional control with Melphalan, complete remission is achieved in about 40% of patients (pt), with frequent toxicity, causing significant short term disability in many and long term incapacity in a few. Since 1989, several studies report the success of the association of Fotemustine and Dacarbazine (DTIC) in the systemic treatment of disseminated melanoma, but serious lung toxicity limited its use. In 1995, we introduce a pilot study with systemic DTIC and using Fotemustine as the perfusion agent.

Patients and Methods: Twenty-eight pt (M-6; F-16) in stages IIIA and IIIAB were introduced in this study, making a total of 30 ILP. DTIC in a dose of 400 mg/m2 was administrated 4 hours before ILP and Fotemustine, in a dose of 100-150 mg/m2, was introduced in the arterial line when the subcutaneous temperature reaches 38°C. Drug perfusion lasts for one hour with local temperatures ascending to 40-41°C.

Results: Results were evaluated by: A - Response rate: Complete Response-16 (53,3%), Partial Resp.- 8 (26,7%), Local disease progression - 1 (3,3%); Local disease stabilization - 1(3,3%), N/evaluated - 2 (6,7%), Lost for follow-up - 2 (6,7%); B - Local toxicity (Wieberdink scale): I-30; C - Systemic toxicity (WHO scale): 0 - 13; I - 11; D - Late local toxicity: Fibrosis-3; Epidermolisis - 4.

Conclusion: Treatment was effective, with a response rate similar to that obtained with Melphalan, but with much lower early toxicity. Therefore, this protocol may represent an innovation in local and regional therapy that would be interesting to explore in order to optimise the technical conditions and outcomes.

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P21 ciclin-dependent kinase inhibitor (CKI) polymorphisms and malignant melanoma: a study of susceptibility and an analysis of clinico-pathological parameters

S. Carrilho[1], M. Soares[2], R. Medeiros[1], S. Costa[1], D. Pinto[1], M.L. Augusto[2], J. Machado Lopes[2], L. Pontes[3], M. Ribeiro[3], C. Lopes[1]. *[1] Instituto Portugues Oncologia, Laboratorio de Patologia Molecular - Serviço de Anatomia Patologica, Porto, Portugal; [2] Instituto Portugues Oncologia, Serviço de Oncologia Medica III, Porto, Portugal; [3] Instituto Portugues Oncologia, Serviço Clinica Oncologica I, Porto, Portugal*

Purpose: In malignant melanomas, G1/S checkpoint abnormalities are known to be of significant importance in the development of the disease. In this study, we examine the presence of two polymorphisms in the G1 CKI gene P21, and its association with melanoma risk, stage, recurrence and median age.

Methods: Blood samples were obtained from 124 patients with melanoma, diagnosed and treated at Instituto Português de Oncologia of Porto. Control subjects were 220 healthy individuals. The analysis of the P21 polymorphisms was performed with the RFLP (Restriction Fragment Length Polymorphism) technique.

Results: The polymorphisms were present in 12,9% of the melanoma patients and in 11,4% of the healthy controls (O.R.=1,16; p=0;673). The analysis of the melanoma cases was performed separating the patients by stage (O.R.=1,45; p=0,314), recurrence (O.R.=2,606; p=0,089) and median age (O.R.=1,23; p=0,617). No significant association was observed between any of this variables and the presence of the polymorphisms.

Conclusion: Our results indicate that these P21 polymorphisms may not be involved in the susceptibility and development of melanoma although they have been associated with the development of some types of cancer.

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Tumor thickness as a predictive parameter of occult metastasis in melanoma patients undergoing sentinel node biopsy

M.B. Lens, M. Dawes. *University of Oxford, Centre for Evidence Based Medicine, John Radcliffe Hospital, Oxford, United Kingdom*

Purpose: Sentinel node biopsy is minimally invasive procedure proposed as a diagnostic test to accurately stage nodal basins at the risk for occult metastases. The purpose of this study is to find an evidence regarding the relationship between tumor thickness and the rate of positive SNs and to estimate the power of tumor thickness to determine the likelihood of the presence of occult nodal metastases in melanoma patients stage I and II.

Methods: A systematic search was performed using Medline and Embase through March 2001. A manual reference search and a manual review of specialty journals also were performed. Our search was restricted to studies published in English language. Of 417 identified studies on sentinel node biopsy in melanoma, 22 studies met our inclusion criteria of whom 12 were included in the analysis.

Results: We summarised results from 12 retrieved studies. Total number of patients undergoing sentinel node biopsy for melanoma was 4218. An occurrence rate of SN metastasis was 17.8% (95% 16.7 to 19.0). The incidence rate of tumor positive SNs increases with tumor thickness: it was less than 1% for lesions <0.75 mm, 8.3% for 0.76-1.50 mm lesions, 22.7% for 1.51-4.0 mm lesions and 35.5% for lesions >4.0 mm in thickness. Statistical test for trend confirmed a strong positive correlation between tumor thickness and SN positivity.

Conclusions: There is a strong evidence that the tumor thickness has significant power to predicy metastasis in SNs in melanoma patients. The

risk of melanoma metastasizing to the regional SNs is proportionally related to Breslow's thickness of primary tumor: as tumor thickness increased, so did the rate of SNs involvement.

However, sentinel lymph node biopsy requires further research.

Soft tissue and bone tumours

300 POSTER

Ewing tumor as second malignancy-the EI(CESS)-experience

A. Wagner-Bohn, S. Ahrens, M. Paulussen, B. Froehlich, M. Kuhlen, H. Juergens. *University of Muenster, Paediatric Hematology/Oncology, Muenster, Germany*

Purpose: Ewing Tumors (ET) have only occasionally been described as second malignancies. Associations of a second ET (sET) to the entity/site of the primary malignancy (pM), to genetic predispositions and to previous treatment remain speculative.

Patients and Methods: The database of the EI(CESS) and the ongoing Euro-Ewing99 trials was searched for patients with sET. The incidence of sET in comparison to pET was compiled, survival analyses were performed and outcome of sET is compared to outcome of pET.

Results: 15/1778 registered patients with sET were identified, with a median age at pM of 6.9y, of sET 17.4y, median time interval to sET was 7.1y. 9/15 patients were < 15y at diagnosis of pM. An estimated annual risk for childhood cancer patients to develop sET of approx. 2,3/105/y is calculated. Considering the heterogeneity of pM in the reported childhood cancer patients (ALL n=3, retinoblastoma (RB) n=2, NHL n=1 osteosarcoma (OS) n=1 RMS n=1), unilateral RB with typical deletion (13q14) is overrepresented (2/9), especially in view of the low relative frequency of primary RB. 5/15 sET developed within the previous irradiated region (n=3 > 40 Gy, n=2 12 Gy) after a median time interval of 7.1y.There was no obvious impact of pM chemotherapy (11/15 pts on various regimens) on the occurrence of sET. The characteristics and the outcome of sET after a median observation time of 34 months are similar to the data in pET.

Conclusion: The cumulative risk for sET is lower than for secondary OS (0,045% vs. 0,15%). Unilateral RB as pM are disproportionaly frequent. Germline mutations were only observed in RB. Radiotherapy may also contribute to the risk of developing not only sOS but sET as post-irradiation sarcomas. The time interval between first and second malignancy is similar to the period reported in other second sarcomas. The prognosis of sET is comparable to pET as long as appropriate chemotherapy is applied.

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301 POSTER

A biopsy of a suspected soft tissue sarcoma in the retroperitoneal space; the diagnostic yield and the risk of contamination of the different procedures

T. van Dalen, F. van Coevorden, A. van Geel, H. Hoekstra, A. Hennipman. *The Dutch Soft Tissue Sarcoma Group, Utrecht, The Netherlands*

Purpose: A biopsy is important to clarify the nature of a retroperitoneal mass, but its value in case of a suspected retroperitoneal soft tissue sarcoma (RSTS) is unclear. The diagnostic accuracy and the influence on the occurrence of local tumour spread of the different biopsy procedures was assessed.

Methods: Data were collected on 143 patients (64 males and 79 females, median age 60 years) in the Netherlands in whom a RSTS was confirmed histologically between 1-1-1989 and 1-1-1994. Biopsies were done during clinical work-up in 85 patients (59%), and in them the yield was assessed of fine-needle aspiration (FNA), core needle biopsy (CNB), and surgical biopsy (SB). The risk of developing local tumour spread was evaluated by comparison of the biopsied patients to those who had no biopsies prior to surgery (n=58).

Results: A total number of 122 biopsies was performed: FNA (n=46), CNB (n=61), and SB (n=25). The proportion of affirmative biopsies was 22% for FNA, 54% for CNB, and 72% for SB (FNA vs. CNB, p=0.001; FNA vs. SB, p<0.001; CNB vs. SB, p=ns). At the time of surgical treatment (n=123), no significant differences in the presence of local tumour spread were seen following preoperative SB (4/16=25%), needle biopsies (8/49=16%), or when preoperative biopsies weren't taken (11/58=19%; p=0.74). Following complete tumour resection (n=78), no significant differences were seen in 5-year local disease free proportional survival (SB, 50%; needle biopsy, 52%, no biopsy, 45%; p=0.91).

Conclusion: The yield of a biopsy in case of a RSTS was limited for all three techniques, being lowest for FNA. No effect of needle and open surgical biopsies was found on the occurrence of locoregional tumour spread.

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Prognostic value of initial management in localized osteosarcoma. A monocentric retrospective analysis

G. Delepine[3], F. Delepine[2], S. Alkallaf[1], E. Guikov[1], N. Delepine[1]. *[1]Hop. Avicenne, 125 rue Stalingrad, 93009 Bobigny; [2]5 Passage du Bon Pasteur, 76000 Rouen; [3]8 rue E. Varlin, 93700 Drancy, France*

Introduction: Many reports attempt to identify the factors which may affect the prognosis in osteosarcoma. We wanted to determine whether the technique of biopsy and/or the initial management could be prognostic factors of long term survival.

Patients: 139 patients (88 males and 51 females aged 4–58 years) with localized high grade osteosarcomas of the limbs were treated and/or followed up by our team between 1984 and 1998. 75 first hand patients had the biopsy performed by the surgeon of the team after local evaluation of the tumor and planning of future en bloc resection. The 84 other patients were referred to us after biopsy or/and induction therapy. No significant differences in initial prognostic factors were observed between the two groups.

Method: All patients received preoperative and postoperative chemotherapy according to the current protocols at the time of their treatment.

3 patients (all referred patients) were primarily amputated. All the other were treated by limb salvage even for fractured or huge tumors and in very young patients. All patients were followed up by their surgeon and their chemotherapist every 3 months during 2 years, then every 6 months for 2 other years and yearly thereafter.

Results: With a median follow up of 10 years (maximal 16 - minimal 2) 12 local recurrences were observed: 10/84 (12%) in referred patients and 2/75 (2.6%) in first hand patients. 54% (46/84) of referred patients are alive in complete remission compared to 73% (40/55) of first hand patients (93% for first hand patients treated by our protocols since 1/1986). In multivariate analysis, the difference is significant (p < 0.02)

Conclusion: Initial management by an experimented team is of crucial importance in long term survival of patients with localized high grade osteosarcoma of the limb. When the diagnosis of osteosarcoma can not be excluded on prebiopsy medical imaging of bone tumor, the patient should be referred, before biopsy, to team experimented in bone tumor oncology.

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A phase I/II clinical trial of Carbon-ion therapy for patients with bone and soft tissue sarcomas not suited for surgical resection

T. Kamada[1], H. Tsujii[1], H. Tsuji[1], T. Yanagi[1], J. Mizoe[1], T. Miyamoto[1], S. Morita[1], H. Kato[1], S. Yamada[1], A. Tateishi[2]. *[1]Natinal Institute of Radiological Sciences, Research center hospital of charged particle therapy, Chiba, Japan; [2]Teikyo university, orthopedic surgery, Tokyo, Japan*

Purpose: To determine the maximum tolerated total dose of that can be delivered by carbon-ion beam irradiation, as defined by the acute toxicity, and to evaluate the effect of carbon-ion beam on the bone and soft tissue sarcomas. This is the first report of carbon-ion treatment of the bone and soft tissue sarcomas.

Methods and Materials: Between April 1996 and February 2000, 64 lesions in 57 patients with the bone and soft tissue sarcomas not suited for surgical resection received carbon-ion treatment. Tumor Sites included mobile spine or para spine in 19 cases, pelvis in 32 cases and extremities in 6. The applied dose was escalated from 52.8 to 73.6GyE(Gray equivalent) in 16 fractions over 4weeks(3.3 to 4.6GyE/fraction). The median tumor size was 559cm3 (range, 20~2290cm3). The minimum follow-up period of the survivors was 15months.

Results: Seven of 17 patients treated with the dose of 73.6GyE experienced RTOG grade3 acute skin reaction. We stopped the dose escalation at this dose level. No other severe reactions (Grade3~) were observed in this series. The 5-year actuarial local control rate for the whole group is 72%. The overall 5-year actuarial survival rate is 46%.

Conclusion: Heavy - ion treatment using carbon-ions appears to be safe and effective in the management of the bone and soft tissue sarcomas not suited for surgical resection, and it will provide better local control and may offer a survival advantage in these patients.