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

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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

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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

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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

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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