**Methods:** miRNAs was produced from normal human epidermal melanocytes (NHEM) and five cell lines of malignant melanoma, and hybridized to a commercial miRNA array. A supervised analysis was performed to compare the expression patterns of miRNAs between two NHEM miRNA replicate samples and five different melanoma samples.
**Results:** 58 miRNAs were found to be significantly altered between the two groups (normal vs. malignant), out of which 57 miRNAs were significantly down-regulated or absent in the melanoma cells relative to control cells, and only one was significantly up-regulated.
Out of the 57 miRNAs that were down-regulated or absent in the melanoma samples, 38 miRNAs belonged to 8 known miRNA clusters, namely to groups of miRNAs that are thought to belong to one regulatory unit of expression. Of these, 27 miRNAs belonged to four clusters that mapped to chromosome 14. Three of these clusters were found to be in very close proximity to one another along ~40 kb of the chromosome.
**Conclusions:** Our observations suggest that aberrations of miRNA expression in this short chromosomal locus may have a role in the pathogenesis of melanoma. This chromosomal region has not been implicated in melanoma thus far. It is yet to be determined whether the absent expression of miRNAs is this region is due to a chromosomal deletion or epigenetic silencing. Although preliminary, our results will hopefully shed light on the role of miRNAs in malignant melanoma, thus providing new potential therapeutic targets.

**9320** POSTER

**Treatment with intravenous High Dose Interferon (HDI) is able to reduce levels of circulating regulatory T (Treg) cells in melanoma patients**

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**Background:** T regulatory (Treg) cells control autoimmunity through "dominant tolerance". Natural Treg cells represents approximately 5−10% of the total CD4+ T cell population, expressing high levels of surface CD25 (high-affinity IL-2a receptor subunit), CTLA-4, and glucocorticoid-induced tumor necrosis factor-a receptor (GITR). Tregs have been shown to be present in tumor and tumor-draining lymph nodes, acting as a potential inhibitory population blocking or "balancing" effector cell function. Thus, depletion of Tregs or blockade of Treg function might be able to enhance antitumor immunity. Recent evidence has been reported about the possibility of High Dose Interferon (HDI) to act through an indirect immunomodulatory rather than a cytotoxic mechanism: a) correlation with the development of autoimmunity (Gogas, NEJM 2006); b) endotumoral increase of CD11c+/CD3+ cells and decrease of CD83+ cells in clinical responders. Therefore, we started a study to verify if iv HDI treatment in melanoma patients could be able to reduce the number of Treg cells in peripheral blood.
**Patients and Methods:** Analysis was performed on melanoma patients referring to the National Cancer Institute of Naples since July 2006 and who addressed to Neoadjuvant or Adjuvant treatment with iv HDI (20 million units/$m^2$, 5 days per week) for 4 weeks. Peripheral blood mononuclear cells (PBMC) were obtained from 22 consecutive melanoma patients. Blood draw was performed at days 0, 8, 15, 22 and 29. PBMC were thawed and labeled with anti-CD4-PerCP and anti-CD25-Pe (IL-2R1) (BD, San Diego, CA) and anti-FoxP3-FITC (PCH101) (eBioscience, San Diego, CA). Labeled cells were analyzed using a FACScalibur (Becton Dickinson).
**Results and Discussion:** Fifteen (68.2%) out of 22 patients showed a decrease of Treg cells in peripheral blood. The average value at day 0 for circulating Treg cells (cTreg) was 2.7%. The average percentage at day 29 was 1.4%. The average reduction was 1.4 (50% reduction in the average value of cTreg). Statistical analysis showed an average decrease of 0.29% per week of treatment. Despite of this clear trend in reducing cTreg by HDI induction treatment, statistical significance was not reached (probably due to the power of the study). Moreover, it has been observed great differences between the disease status, the prognosis (recurred/not recurred pts, alive/deceased) and an increased basal percentage of cTreg in PBMC. Our preliminary data are consistent for an effect of HDI on reducing circulating Treg cells, although no conclusion about the role of such reduction in terms of response to treatment or as prognostic markers of better/worse disease can be inferred. Further data are awaited in order to verify if the Treg reduction after a HDI treatment may indeed contribute to the antitumor response.

**9321** POSTER

**Metastatic uveal melanoma, clinical characteristics and survival: a single center experience on 58 patients**

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**Background:** Uveal melanoma is a rare disease. Metastases develop in 6.5−35% of the patients, most commonly in the liver. In general the survival of metastatic uveal melanoma is poor, with a median survival of 5 to 7 months. The aim of this study is to asses clinical characteristics and survival in patients with metastatic uveal melanoma.
**Methods:** We reviewed retrospectively all patients with metastatic uveal melanoma diagnosed between 1983 and end 2008 at our institution.
**Results:** We analyzed a total of 58 patients (24 male and 34 female) with a median age of 61 years (31−84). Primary tumor was localized in 89.7% in choroids, 24 patients were treated with surgery (79.2% ennucleation and 20.8% partial resection) and 33 with braquitherapy. The median time for the development of metastases was 25.63 months (0.17−102.43) and 56 patients had hepatic involvement, bilobar on 63.8% of the cases and with more than 8 hepatic lesions on 51.7%. In sixteen patients (27.6%) there were two or more sites involved. Six patients (8.6%) were treated with surgery (segmentectomy and lobectomy), 5 of them had recurrence of the liver disease (median time to recurrence 11 months); 2 patients (3.4%) were treated by radiofrequency; 24 patients (41.4%) received systemic chemotherapy (56.5% Dacarbacine and 17.4% Fotemustine); and 16 (27.6%) the best supportive care. With a median follow up of 7 months, the median overall survival (OS) for the total of the patients was 10.83 months (6.92−14.74; 95% CI). Patients with local metastatic treatment (surgery and radiofrequency) were not assessable for individual OS. For patients who did chemotherapy median OS survival was 10.83 months (5.35−16.308; 95% CI) and the patients without treatment had an OS of 8.033 months (2.46−13.61; 95% CI), There were more patients with characteristics associated with poor survival such as worst ECOG and elderly patients in the group without treatment.
**Conclusions:** Our results are similar to the published data and confirm again that uveal melanoma relapse is more common on the liver and has a poor prognosis. Due to diffuse liver involvement only a few number of patients were elegible for local metastatic treatment according to our hepatic surgery committee criteria. Despite different treatment options the overall survival was poor. Heterogenicity of this patients group does not allow to individualize prognostic factors.

**9322** POSTER

**Novel protein kinase inhibitors in melanoma**

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**Background:** Systemic therapy has a very limited effect on survival of patients with metastatic melanoma, and the prognosis remains very poor. Therefore there is an urgent need to identify new therapeutic targets that may improve response. The aim of this study was to screen a library of 160 protein kinase inhibitors in melanoma cell lines and to select the most effective of inhibitors and their targets for further evaluation as novel therapeutic approaches for metastatic melanoma.
**Methods:** The InhibitorSelect™ Library (Merck) consists of a 160 protein kinase inhibitors (10 mM). Screening was performed on two melanoma cell lines; Sk-Mel-28 (BRAF mutant) and Sk-Mel-2 (NRAS mutant). Each inhibitor (1 μM) was tested in triplicate, in both cell lines. Proliferation was assessed using the acid phosphatase assay following a 5 day incubation period. $IC_{50}$ values were determined for selected inhibitors by performing dose response assays.
**Results:** Of the 160 protein kinase inhibitors, 20 and 29 compounds achieved $\geqslant$50% growth inhibition in the Sk-Mel-28 and the Sk-Mel-2 cell lines, respectively. Six inhibitors achieved 20−49% inhibition in the Sk-Mel-28 cell-lines, while 10 compounds achieved this level of inhibition in the Sk-Mel-2 cell-line. The 20 compounds which achieved $\geqslant$50% growth inhibition in the Sk-Mel-28 cell line also achieved $\geqslant$50% growth inhibition in the Sk-Mel-2 cell line. The effective inhibitors included a number of cyclin dependent kinase (Cdk) inhibitors (Table 1) and inhibitors of the PI3K/Akt/mTOR pathway. Two Cdk inhibitors were selected for further analysis of $IC_{50}$ values in a panel of melanoma cell lines.
**Conclusions:** We have identified 20 protein kinase inhibitors which inhibit proliferation of in two melanoma cell lines, which represent models of BRAF

and NRAS mutated metastatic melanoma. Two Cdk inhibitors, which target Cdk2/Cdk4 and Cdk4/Cdk6, have been selected for further analysis to evaluate these cyclin dependent kinases as novel therapeutic targets for the treatment of metastatic melanoma.

Table1: Effective Cdk inhibitors in two melanoma cell lines

| Inhibitor | Sk-Mel-28% Inhibition (± std dev) | Sk-Mel-2% Inhibition (± std dev) |
|---|---|---|
| Alsterpaullone, 2-Cyanoethyl (Cdk1/5; GSK-3b) | 65.6± 13.0 | 64.5± 4.1 |
| Cdk1/2 Inhibitor III | 99.9± 0.0 | 98.0± 1.0 |
| Cdk4 Inhibitor III (Cdk2/4) | 72.6± 22.8 | 53.9± 8.5 |
| Cdk/Crk Inhibitor | 99.8± 0.0 | 99.6± 0.2 |
| Fascaplysin (Cdk4/6) | 99.2± 0.6 | 98.0± 1.0 |

**9323** POSTER

**Expression of cancer-testis antigens is a poor prognostic factor in primary but not metastatic melanoma**

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**Background:** Cancer-Testis Antigens (CTAg) are commonly expressed in melanoma. Their immunogenicity and limited normal tissue expression make them attractive targets for anti-cancer immunity. Using three CTAg against which vaccines have been developed, we evaluated whether CTAg expression is a prognostic factor in cutaneous primary and metastatic melanoma.
**Methods:** Expression of MAGE-A1, MAGE-A4 and NY-ESO-1 by immunohistochemistry was evaluated in 233 stage II (primary) and 261 stage III/IV (metastatic) melanoma samples. Univariate analysis (UVA) was performed by the Kaplan-Meier method using the log-rank test. Multivariate analysis (MVA) using CTAg expression and known prognostic factors was performed by the Cox proportional-hazards regression model.
**Results:** Expression of at least 1 CTAg (CTAg+ve) was a poor prognostic factor in stage II melanoma, with median relapse-free survival (RFS) of 72 months for CTAg-ve tumours *vs.* 45 months for CTAg+ve tumours ($p = 0.008$). On UVA, CTAg expression, ulceration, Breslow thickness and mitotic rate were identified as prognostic factors. MVA demonstrated that the prognostic impact of CTAg expression in primary melanoma was comparable to ulceration and Breslow thickness, both currently accepted prognostic factors (Table). CTAg expression was not a prognostic factor in stage III/IV melanoma with median overall survival of 23 months each for CTAg+ve and CTAg-ve tumours ($p = 0.72$). The prognostic significance of tumour stage, performance status (PS) and LDH, all of which are known prognostic factors in metastatic melanoma, was confirmed on UVA and MVA (Table).

Cox proportional-hazards regression analysis

| Parameter | Subgroup | p-value | Hazard ratio (95% confidence interval) |
|---|---|---|---|
| Stage II (primary) melanoma | | | |
| Any CTAg | positive or negative | 0.010 | 1.72 (1.14−2.58) |
| Ulceration | Present or absent | 0.008 | 1.80 (1.17−2.77) |
| Breslow thickness | >4.0 mm or <4.01 mm | 0.003 | 1.87 (1.24−2.81) |
| Mitotic rate | $\geqslant 4\,mm^2$ or $0\,mm^2$ | 0.14 | 1.91 (0.82−4.44) |
| Stage III/IV (metastatic) melanoma | | | |
| Any CTAg | positive or negative | 0.71 | 0.93 (0.64−1.36) |
| Stage | IV or III | 0.02 | 1.60 (1.08−2.38) |
| PS | ECOG 2+ or 0−1 | <0.001 | 3.27 (1.80−5.94) |
| Serum LDH | High or normal | <0.001 | 2.64 (1.72−4.04) |

**Conclusion:** CTAg expression was a significant prognostic factor in primary but not metastatic melanoma. The prognostic impact of CTAg expression in cutaneous primary melanoma was comparable to Breslow thickness and ulceration. Further study into CTAg function and the impact of clinical targeting is warranted.

**9324** POSTER

**Radical radiotherapy for clinically localized sebaceous carcinoma of the eyelid: a retrospective analysis of 78 patients**

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**Background:** To analyze retrospectively the results of radiotherapy for clinically localized sebaceous carcinoma of the eyelid.
**Materials and Methods:** The records of 78 patients with histologically confirmed sebaceous carcinoma who were treated between 1983 and 2007 were reviewed. Patients with distant metastasis and/or lymph node metastasis at initial diagnosis were excluded in this study. All 78 patients were treated with radical radiotherapy to the primary tumor (electron beams: 74 patients; X-ray: 4 patients) and the total radiation doses ranged from 30.0 to 70.4 Gy (median: 60.0 Gy). The median follow-up of all patients was 64.8 months (range, 2.8−270.3 months). Overall survival (OS), disease-free survival (DFS) and local control (LC) rates were calculated actuarially according to the Kaplan-Meier method, and differences between groups were estimated using the log-rank test. Multivariate analysis was performed using the Cox regression model.
**Results:** At the time of analysis, 10 patients (12.8%) died, and local recurrence was observed in 31 patients (39.7%). The 5-year actuarial OS, DFS and LC rates for all patients were 89.6%, 54.3% and 58.4%, respectively. Patients with T1−2 tumors had a significantly higher LC (5-year LC: 74.2%) than those with T3−4 tumors (5-year LC: 40.0%; $p = 0.014$). Multivariate analysis indicated that T stage alone was a significant prognostic factor for LC. Concerning DFS, patients with T1−2 tumors had a significantly higher DFS (5-year DFS: 71.5%) than those with T3−4 tumors (5-year DFS: 34.1%; $p = 0.0071$). Multivariate analysis indicated that T stage alone was a significant prognostic factor for DFS. Late morbidity of CTCAE Grade 3 was observed in only 1 patient (eyelid dysfunction).
**Conclusions:** These results indicate that radical radiotherapy is the treatment of choice for early-stage (T1−2) sebaceous carcinoma of the eyelid. On the other hand, multimodal treatment may be recommended for advanced (T3−4) disease.

**9325** POSTER

**Efficacy of a hypofractionated schedule in electron beam radiotherapy for epithelial skin cancer: analysis of 434 cases**

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**Background:** To evaluate the efficacy of radiation therapy for epithelial skin cancer and compare treatment outcomes of two electron beam fractionation schedules.
**Material and Methods:** Outcome data of 434 epithelial skin cancers in 333 patients, treated between 2001 and 2006 were analysed. 332 were basal cell carcinomas (BCC) and 102 squamous cell carcinomas (SCC). Patients were treated with electron beam irradiation for primary skin cancer ($n = 386$) or after previous resection ($n = 48$), and received either 54 Gy in 18 fractions ($n = 159$) or 44 Gy in 10 fractions ($n = 275$), 4 fractions per week. A pilot study was performed to evaluate cosmetic outcome in 14 patients. Local recurrence free rates (LRF) were analysed using the competing risk method. Secondary endpoints were metastases free rates, cancer specific survival (CSS) and cosmetic result.
**Results:** Median follow up was 42.8 months. Actuarial 3-year LRF rates were 97.5% and 96.1% for 54 Gy and 44 Gy, respectively. For BCC, 3-year LRF rates were 97.6% for tumours treated with 54 Gy and 96.9% for those treated with 44 Gy. In SCC 3- year LRF rates were 97.0% for 54 Gy and 93.6% for 44 Gy (n.s.). T stage was found to be a significant factor for recurrence ($p = 0.036$). No significant differences in LRF rates were found for fractionation schedule, age, histology, primary or postsurgical treatment, or tumour location. In 5 patients with SCC metastases were diagnosed. Three-year CSS was 98% for SCC and 100% for BCC. Cosmetic outcome was scored 'good' or 'fair' by 75% of patients and 87% of objective observers in the 54 Gy group and 100% and 83% in the 44 Gy group.
**Conclusions:** Electron beam irradiation is a safe and effective treatment modality for epithelial skin cancer, and can be recommended both for primary treatment in cosmetically sensitive areas, and after surgery in case of involved margins. Local control rates greater than 95% are found for T1 and T2 tumours. In view of the similar efficacy and patient convenience of the hypofractionated schedule, 44 Gy in 10 fractions can be regarded the radiation schedule of choice, especially in elderly patients.