**9316** POSTER

**Follow-up study in high-risk uveal melanoma patients**

P. Mariani[1], V. Servois[2], S. Piperno-Neumann[3], J. Couturier[4], C. Plancher[5], C. Levy-Gabriel[1], L. Lumbroso Le Rouic[1], R. Salmon[1], B. Asselain[5], L. Desjardins[1]. [1]*Institut Curie, Surgery, Paris, France;* [2]*Institut Curie, Radiology, Paris, France;* [3]*Institut Curie, Oncology, Paris, France;* [4]*Institut Curie, Tumor biology, Paris, France;* [5]*Institut Curie, Biostatistics, Paris, France*

Uveal melanomas have a significant predilection for metastasis to the liver: up to 50% of patients will develop metastases, the liver is the sole or dominant site in more than 80% of them. Microscopic complete (R0) surgical resection of liver metastases improves survival to 22 months in very high selected patients. Despite aggressive therapy, survival is poor, the 1-year survival rate is 10%. Prognostic factors from the primary include tumor diameter and thickness, anterior location, extraocular extension, epithelioid cell type, monosomy 3 and gene expression profile. Early identification of a high-risk group of patients might allow early detection of metastases, and increase R0 liver surgery.

We began in October 2006 an intensive follow-up prospective study to detect early minimal lesions with liver MRI in asymptomatic high risk patients. High-risk was defined by thickness >8 mm or diameter >15 mm, or extrascleral extension, or monosomy 3. Primary objective was to increase R0 liver resection rate from 10 to 30% ($\alpha$ risk = 0.04 and $\beta$ risk = 0.05); secondary objectives were overall survival, metastasis-free survival, predictive value of MRI and liver functional tests (LFT's). After treatment of the primary, patients undergo liver MRI and serum samples/6 months, LFTs/3 months. MRI screening consists in T1, T2, T1 dynamic series with gadolinium injection and LFT's in total bilirubin, ALAT/ASAT, Alkaline phosphatase, GGT, LDH. MRI suspect abnormalities lead to surgical procedure. Information document is sent to radiologists, MRI central review is conducted by Institut Curie radiologists.

From Oct 2006 to March 2009, 85 patients were enrolled, median age 60 (32−83), sex ratio M39/F46. The median uveal tumor diameter was 19 mm (11−26), median thickness 11.6 mm (2.7−17), with retinal detachment in 65 patients, and extraocular spread in 7. Local primary treatment consisted in proton beam therapy in 9 patients, enucleation in 76. Secondary enucleation was performed in 5 cases (2 for local relapse). The histological cell type was epithelioid in 18 cases, fusiform in 17, mixed in 41. Monosomy 3 (FISH) was present in 41/58 analysed enucleations. With a median follow up of 15 months, the metastasis-free survival is 72%. 2 Patients underwent enucleation for local relapse. 24 patients developed metastasis, 2/11 operated patients had R0 liver surgery (18%). To date, 6 patients died (5 from metastasis, 1 myocardial infarction) and 17 patients are alive with metastasis.

MRI and LFT's screening analysis will be presented with an updated follow up in the 85 available patients.

**9317** POSTER

**Ipilimumab re-induction after progression in patients with advanced melanoma enrolled in Phase II clinical trials**

K. Harmankaya[1], D. Minor[1], G. Linette[1], R. Ridolfi[1], j. Corsa[1], R. Ibrahim[1], C. Lebbé[1]. [1]*Medical University of Vienna, Vienna, Austria*

**Background:** Ipilimumab is a monoclonal antibody that activates an antitumor immune response by blocking cytotoxic T-lymphocyte antigen-4. The safety and efficacy of ipilimumab at 10 mg/kg given in an induction/maintenance regimen to patients (pts) with advanced melanoma have been evaluated in completed Phase II clinical trials. In the ongoing rollover study presented here, pts with disease control (DC) were offered re-induction with ipilimumab at 10 mg/kg upon progressive disease (PD). The safety and efficacy of re-induction was assessed as one objective of this rollover study.

**Methods:** In the Phase II clinical program (CA184−007, 008, -004, and MDX010−15), ipilimumab was administered every 3 weeks (Q3W) x 4 (induction) and pts treated with ipilimumab at 10 mg/kg who achieved DC [complete response, partial response, or stable disease] could be re-induced with ipilimumab at 10 mg/kg in the rollover study, CA184−025, after PD. The exception was study 022 (based on the use of lower doses in 2 arms of the study), where any pt with PD was eligible for re-induction in study 025. The pts rolled over to study 025 were re-induced with ipilimumab at 10 mg/kg immediately upon enrollment. Pts who achieved DC and entered the maintenance phase of a parent trial continued maintenance therapy with ipilimumab at 10 mg/kg (Q12W) in study 025, and received re-induction with ipilimumab at 10 mg/kg upon PD. Any pt who did not achieve DC following re-induction, withdrew, or had unacceptable toxicity requiring discontinuation of ipilimumab, entered a follow-up phase.

**Results:** We present 49 pts treated with ipilimumab at 10 mg/kg in a parent study who were re-induced with ipilimumab at 10 mg/kg in study 025 following initial DC and subsequent PD; 35 of 49 pts were re-induced with ipilimumab at 10 mg/kg immediately upon enrollment in study 025. Of pts that entered the maintenance phase in study 025, 14 have been re-induced with ipilimumab at 10 mg/kg following PD. Objective response and/or disease stabilization after re-induction were observed. Preliminary data showed safety and efficacy of retreatment is comparable to what was observed at first induction. Data collection is ongoing.

**Conclusions:** Data on the activity and safety of ipilimumab during re-induction will be of special interest for the long-term management of advanced melanoma pts. Details of the final analyses will be presented at the meeting.

**9318** POSTER

**Clinical and histopathological risk factors to predict sentinel lymph node positivity, disease free and overall survival in clinical stage I-II AJCC skin melanoma – a prospective study**

M. Mandalà[1], G.L. Imberti[2], D. Piazzalunga[3], M. Belfiglio[4], R. Labianca[5], M. Barberis[6], L. Marchesi[7], T. Motta[6], L. Novellino[8], C. Tondini[9]. [1]*Ospedali Riuniti bergamo, Unit Medical Oncology Department Oncology-Hematology, Bergamo, Italy;* [2]*Ospedali Riuniti bergamo, Unit of dermatology, Bergamo, Italy;* [3]*Ospedali Riuniti bergamo, Unit of Surgery, Bergamo, Italy;* [4]*Consorzio Mario Negri Sud, Department of Clinical Pharmacology and Epidemiology, S. Maria Imbaro, Italy;* [5]*Ospedali Riuniti Bergamo, Department of Oncology and Hematology, Bergamo, Italy;* [6]*Ospedali Riuniti Bergamo, Department of Pathology, Bergamo, Italy;* [7]*Ospedali Riuniti Bergamo, Department of Dermatology, Bergamo, Italy;* [8]*Ospedali Riuniti Bergamo, Department of Surgery, Bergamo, Italy;* [9]*Ospedali Riuniti Bergamo, Unit Medical Oncology, Bergamo, Italy*

**Background:** The aim of the study was to investigate clinical and pathological risk factors to predict the sentinel node positivity (SLN), the disease free (DFS) and overall survival (OS) in clinical stage I-II AJCC primary cutaneous melanoma (PCM) through a prospective mono-institutional data base.

**Materials and Methods:** The study included consecutive patients with PCM, all diagnosed, treated and followed up prospectively by a multidisciplinary team. Logistic regression was used to investigate the association between DFS, OS, SLN positivity and Breslow thickness, Clark level, tumor infiltrating lymphocytes (TIL), ulceration, lesion site, gender, regression and age.

**Results:** From November 1998 to December October 2008, 1251 patients with PCM were referred to the Bergamo Riuniti Hospital. The median age was 51.7±16.6 years; 32.2% (N = 393) with > 60 years, male: 45%. Among those 394 patients with primary vertical growth phase (VGP) melanoma and no clinical evidence of metastatic disease who underwent SLN biopsy. In all, 74 of the 394 patients had 1 positive SLN (18.8%). In the multivariate analysis, no extremity primary (extremity vs axial = truncal and headh/neck) OR 0.44, 95% CI 0.22−0.89, $p < 0.023$), TIL (TIL vs no TIL 0.46, 95% CI 0.24−0.88, $p < 0.02$), and thickness (>4 mm vs 1.1−2 mm OR 25, 95% CI 4.95−126.32) predicted SLN positivity. A multivariate stepwise analysis confirmed these results. The histologic status of the SLN was the most significant predictor of DFS and OS. Patients with a negative SLN had a 5-year DFS of 75.9%, compared with 35.2% in patients with a positive SLN ($p < 0.0001$) and a 5-years OS of 88.7% versus 42.9% respectively ($p < 0.0001$).

**Conclusions:** Our study demonstrate that the absence of TILs predicts SLN metastasis, at multivariate analysis only the SLN positivity predicts DFS and OS.

**9319** POSTER

**Alterations in the expression patterns of micro-RNAs during the transformation process of normal human melanocytes into malignant melanoma**

R. Leibowitz-Amit[1], D. Avni[2], Y. Sidi[2]. [1]*Sheba Medical Center, Oncology Institute, Tel Hashomer, Israel;* [2]*Sheba Medical Center, Department of Medicine C, Tel Hashomer, Israel*

**Background:** Malignant melanoma is a devastating disease with a constantly increasing incidence worldwide. Metastatic melanoma is almost inevitably fatal, with current treatment options being highly disappointing. Although this disease has been extensively studied, little is known about the molecular mechanisms underlying melanocyte transformation. Recently, a novel regulatory mechanism of gene expression has been discovered, based on the cellular generation of short RNA sequences termed micro-RNAs (miRNAs). miRNAs can silence or decrease the expression of genes within cells. Many lines of evidence indicate that miRNAs have an important role in cancerous transformation. Our work is aimed at studying the role of miRNAs in the pathogenesis of melanoma.

**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**

P. Ascierto[1], G. Gentilcore[1], M. Napolitano[2], E. Simeone[1], M. Capone[1], A. Daponte[1], G. Palmieri[3], G. Castello[2], E. Celentano[4], N. Mozzillo[5]. *[1]Istituto Nazionale Tumori Fondazione Pascale, Medical Oncology Unit, Napoli, Italy; [2]Istituto Nazionale Tumori Fondazione Pascale, Immunology Unit, Napoli, Italy; [3]Istituto di Chimica Biomolecolare - CNR, Tumor Genetics Unit, Sassari, Italy; [4]Istituto Nazionale Tumori Fondazione Pascale, Epidemiology Unit, Napoli, Italy; [5]Istituto Nazionale Tumori Fondazione Pascale, Skin Sarcoma Head & Neck and Thyroid Cancer Department, Napoli, Italy*

**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**

M. Plana[1], F. Pons[1], I. Fernandes[2], F.J. Perez[3], J. Pera[4], J.M. Caminal[5], L. Jiménez[1], X. Garcia Del Muro[1], J. Piulats[1]. *[1]Institut Català d'Oncologia, Medical Oncology, L'Hospitalet. Barcelona, Spain; [2]Hospital Santa Maria, Medical Oncology, Lisbon, Portugal; [3]Institut Català d'Oncologia, Clinical Research Unit, L'Hospitalet. Barcelona, Spain; [4]Institut Català d'Oncologia, Radiotherapy Oncology, L'Hospitalet. Barcelona, Spain; [5]Hospital Universitari de Bellvitge, Ophtalmology, L'Hospitalet. Barcelona, Spain*

**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**

T. Mahgoub[1], M. Clynes[1], J. Crown[2], N. O'Donovan[1]. *[1]Dublin City University, National Institute for Cellular Biotechnology, Dublin, Ireland; [2]St Vincent's University Hospital, Dept of Medical Oncology, Dublin, Ireland*

**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