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FIRST WORLD MEETING OF INTERDISCIPLINARY MELANOMA/SKIN CANCER CENTERS

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ABSTRACTS

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A1. In vivo characterization of dermoscopic patterns by confocal microscopy improves melanoma diagnosis

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Our study aimed to identify in vivo cytological and architectural features underlying dermoscopic patterns of melanocytic lesions, and to correlate them with histology. 202 melanocytic lesions (76 melanomas, 114 nevi and 12 Spitz nevi), were recorded by dermoscopy and confocal microscopy. Pigment network was characterized by rings of bright cells surrounding roundish dark areas corresponding to dermal papillae and to regular rete-ridge at histology. Atypical network in melanomas was characterized by irregular in size and shape papillae at confocal microscopy, and disarrangement of architecture at histology. Globules correlated to compact aggregates of cells both at confocal and histology, whereas dishomogeneous aggregates of atypical cells were observed in melanomas. Interestingly, confocal microscopy enabled the exploration of the structures underlying dark diffuse pigmentation, allowing to discriminate between the abundant melanin content in nevi and disarrangement with pagetoid cells in melanomas. Moreover, facing a blue hue confocal microscopy permitted to discriminate between the exclusive presence of inflammatory infiltrate and cyto-architectural atypia. The knowledge of the cyto- architectural aspects of different dermoscopic patterns observable by confocal microscopy may be useful for early melanoma diagnosis.

A2. In vivo microscopic characterization of the dermoscopic blue hue by laser confocal microscopy

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In dermoscopy, the blue hue is a clue for malignancy, although observable in benign lesions. Reflectance-mode confocal microscopy is a novel technique allowing high resolution in vivo visualization of skin structures. We aimed to define in vivo cyto-architectural substrates of blue areas and blue veil for diagnostic purposes. 48 melanomas, 53 common nevi and 21 Spitz nevi showing a blue hue at dermoscopy were studied. Blue areas were predominantly characterized by plump cells corresponding to melanophages and flogosis at histology, whereas blue veil by pagetoid infiltration, atypical cells and non homogeneous nests, consistent with melanoma diagnosis. Whereas blue veil resulted specific but low sensitive for melanoma diagnosis, and it was in almost cases characterized by confocal alteration suggestive of melanoma as well, blue areas were present in similar proportion in melanomas and in nevi. Therefore, confocal microscopy enabled the visualization of cytological substrates of blue areas, evidencing pagetoid cells, architectural disarray and nucleated cells infiltrating dermal papillae in the majority of melanomas. Thus, it is possible to assert that confocal microscopy resulted useful for an accurate diagnosis in lesions presenting blue hue in dermoscopy.

A3. The impact of in vivo reflectance confocal microscopy for the diagnosis of melanoma

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Reflectance confocal microscopy is a new non invasive technique allowing the in vivo evaluation of the skin. The aim of the study was to evaluate the sensitivity and specificity of confocal features for the diagnosis of melanoma. 351 equivocal melanocytic lesions (136 melanomas and 215 nevi) were evaluated for 37 confocal features by two blinded expert observers. Chi-square test, multivariate discriminant analysis and binary logistic regression were performed for the identification of the significant features and for testing different diagnostic models. Epidermal disarray and pagetoid cells in the epidermis, non-edged papillae and cellular atypia at the junction and atypical nests and bright nucleated cells in the upper dermis were mostly observed in melanomas. On the other hand, benign lesions were predominantly characterized by regular dermal-epidermal architecture, and absence of pagetoid infiltration and atypical. Owing to the visualization of cellular aspects, confocal microscopy improved diagnostic specificity and seemed useful for second level examination of equivocal lesions.

A4. Melanoma coinciding with psoriasis: a clinical and histopathological diagnostic challenge

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Psoriasis and dysplastic nevi are prevalent dermatosis in Caucasian population

Material and methods: Clinical, dermoscopy, histopathologic and immunohistochemical features of two cases of melanoma developed in a psoriatic lesion.

Results: First case was a 32 year-old woman affected by dysplastic nevi syndrome and chronic psoriasis. During a psoriasis flare up many melanocytic lesions showed inflammation. On right shoulder one of the lesions presented marked atypia and regression dermoscopic features that was excised with diagnosis of in situ melanoma. Second case: A 23 year-old man with familial history of melanoma, who consulted by a pigmented and recent inflamed lesion on abdomen. On physical examination a debut of guttate psoriasis flare was observed. The suspicious lesion showed target appearance, pigmented in the centre and erythematous on periphery. On dermoscopy it showed melanocytic criteria, symmetry, globular pattern in the centre, and erythema and dotted vessels in the skin around. Since it was referred to change and enlarge in the last months, it was excised. The histopathology showed criteria of in situ melanoma, coexisting with psoriasis features.

Conclusions: Histopathology of psoriasis could imply confusing features to distinguish melanoma from dysplastic nevi since architectural atypia and epidermal changes are presented. This is the second report of melanoma coinciding on psoriatic plaque. Awareness of this possibility could imply an accurate diagnose for dermatologist and dermatopathologist.

A5. Early melanomas on the limbs: clinical, dermoscopical, confocal reflectance microscopy and histopathological findings

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Early melanomas on limbs, especially on lower limbs of women, can be easily misdiagnosed. Dermoscopy has shown an improvement in diagnostic accuracy of clinically difficult melanomas. In vivo confocal reflectance microscopy (CRM) is a non-invasive imaging technique that allows a real time skin examination on horizontally axis with a very high resolution.

Material and methods: We describe a series of 22 cases of early melanomas on the limbs that showed no specific criteria for melanoma on clinical examination. Dermoscopic, CRM and histopathologic correlation of the lesions is described.

Results: All lesions were clinically unsuspicious. On dermoscopy all of them except 4 were asymmetric and showed one or two colours. We could classify them within 4 categories showing: 1. Prominent network with broad lines, 2. Delicate pigment network; 3. Hypopigmented with dotted vessels, 4. Diffuse light pigmentation with perifollicular pigmentation. CMR showed in groups 1 to 3 a prominent pagetoid spreading within epidermis layers, with roundish large cells. In group 4 dendritic melanocytes with follicular infundibule invasion, with mild pagetoid infiltration in epidermis.

Histopathologic examination confirmed the same features seen in CRM images.
Conclusions: The recognition of the dermoscopic features even in the case of lesions with poor pigmentation on the limbs is crucial. MRC reveals a strict correlation with the pathological findings.

A6. Tumour mapping in melanoma. Dermoscopy and confocal reflectance microscopy as additional tools for obtaining fresh tumor sample

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Dermoscopy has improved diagnostic accuracy in tumoral and non tumoral skin diseases. It has been used select partial biopsies and to help histopathologic study.

Objective: the elaboration of a model for the in vivo and ex-vivo mapping of melanocytic tumours.

Methods: a protocol was designed based on the imaging study of the tumor before excision with in vivo dermoscopy and confocal reflectance microscopy, and immediately ex vivo dermoscopy after excision, marking to the pathologist the selected areas of interest. This method allowed the sampling for molecular analyses of the specific areas identified by imaging techniques.

Results: Besides diagnostic interest, dermoscopy and confocal microscopy are useful for the selection of the most representative areas of tumours. Regression structures, invasive and superficial areas, ulceration, tumour vascularization, and tumour margins were the main features studied before excision with a specific histopathologic correlation. Dermoscopy after excision was useful to identify special areas of interest for routine histopathology and for research.

Conclusion: Melanoma can be very heterogeneous and much information is contained in the primary tumour. Imaging techniques may help to obtain critical information with a relevance in diagnosis, prognosis and research. This procedure allows to specifically sampling different areas of the tumour for further histological and/or molecular investigations.

A7. The value of contrast-enhanced ultrasonography in the detection of liver metastases in the follow-up of patients with stages III and IV melanoma

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The objective was to assess the value of contrast-enhanced ultrasonography (CEUS) in the early detection of liver metastases compared with conventional ultrasound (CUS) techniques combined with contrast-enhanced multidetector spiral computed tomography (CEMDSCT) in the follow-up of high-risk melanoma patients. 68 consecutive patients with AJCC stages III and IV melanoma were enrolled in this prospective double-blind study between 2004 and 2006. All patients underwent conventional US, CEUS (Sonovue®) and CEMDSCT. Each examination was interpreted blindly and the combination of CEMDSCT, follow-up, and possible magnetic resonance imaging and biopsy was the gold standard. Standard of reference found 74 liver metastases in 21 patients (31%). 69 liver metastases were detected by CEUS as compared to 47.

A8. Imaging in Primary and Metastatic Cutaneous Melanoma

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The aim of the exhibit is to show the utility of the different radiological (Ultrasound (US), Computed Tomography (CT), Magnetic Resonance (MR)) and Nuclear Medicine techniques in the staging and follow up of cutaneous melanoma from primary tumor to distant metastases. In early stages high frequency US may be used in assessing the depth of the primary tumor and the invasion of locorregional lymph nodes. Nuclear Medicine has become a standard in melanoma staging localizing the sentinel node which is an important prognostic factor. Metastatic melanoma (stage IV) can show characteristic radiological appearance and a wide range of locations. CT, PET/CT and MR play a role in the initial staging of the disease and follow up screening for extralocorregional progression. We report and illustrate our experience in the role of the different imaging techniques in the different stages of the disease focusing on the frequency, the localization and the radiological features of distant metastases.

A9. Littoral cell angioma mimicking a splenal metastase in metastatic melanoma

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We present a case of a 48-year-old male with malignant melanoma stage IV. In routine surveillance he presented two new pulmonal lesions and one new splenal lesion of approximately 10 mm. First he underwent surgery of the pulmonal lesions, revealing metastatic melanoma. In a second surgery the patient was spleenectomized. Astonishingly, the histological finding indicated a littoral cell angioma. A littoral cell angioma is a rare benign tumour of vascular proliferation unique to the spleen. The aetiology is unknown. Conclusion: Regarding our patient, both metastatic disease as well as a benign lesion were present. However the differentiation between may be difficult. Probably further investigations with PET-CT scans may be helpful in such cases.

A10. Measurement of malignant melanoma thickness with high-frequency sonography. Correlation with measurements obtained on histologic specimens

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Objective: the purpose of this study was to determine the accuracy and efficacy of high-frequency sonography in the preoperative measurement of malignant melanoma thickness.

Materials and methods: thickness of 54 primary melanomas after dermoscopic examination was measured using a Sonoline Antares (Simens, Erlangen, Germany) high-frequency US scanner with a 10 MHz linear array trasducer, before surgical resection of the lesions. Sonography measurements were compared with measurements obtained on surgical specimens (Breslow). Correlation coefficient* (CC) between both measurements was calculated as well as sensibility (SE), specificity (SP),diagnostic accuracy (DA), positive predictive value (PPV) and negative predictive value (NPV) of sonography in correctly differentiating between lesions thicker and thinner than 1 mm.

Results: an excellent CC was obtained between presurgical sonography measurement and Breslow (CC: 0.93). SE, SP ,PPV and NPV of sonography in correctly classifying lesions as thicker or thinner than 1 mm were 86%, 97%, 93%, 95%, 91% respectively.

Conclusions: high-frequency sonography provides an accurate preoperative measurement of tumor thickness. Sonography may allow the precise evaluation of the tumor and improve the surgical planning of wide margin excision.

A11. Computer support for standardized dermoscopy report

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The evaluation and diagnosis of melanoma that is widely used all over the world. Recently, standardization efforts of the International Dermoscopy Society have resulted in a proposal for a unified reporting format in dermoscopy for teleconsultation. Based on this proposed standard, we designed and implemented a computer framework that allows structured data entry of lesion reports. The data that can be entered consists of images, patient data, and findings of the examination. Our computer framework contains the following components: A software for data entry, a machine-readable dermoscopy data interchange format, and a means for displaying the data files in a web browser. Using this framework, physicians can record the information gathered during patient examinations in a structured form using a simple computer program. This program produces two kinds of output: A PDF file, which can be printed and kept as a paper record; and a machine-readable XML file, which can easily be stored in a data base, or sent to colleagues for teleconsultation. The conversion of machine-readable XML file to human-readable information is done via an XSL stylesheet, which allows the display of the data and images in a web browser.

A12. Confocal microscopy assistance in the recognition of a difficult lesion of the face

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We report a case of lentigo maligna on the face with negative dermoscopic features and not significantly changes during a 5-year follow-up, ruled out by means of confocal microscopy. A 53 year old patient with a freckle on the nose was examined at our Department. At dermoscopy the lesion was characterized by light brown pseudo network showing a darker pigmentation. Since dermoscopic findings were not conclusive in vivo confocal microscopy was performed to rule out the diagnosis. Evaluating superficial layers, few pleomorphic cells with bright ovalar body and coarse branching dendrites, consistent for malignant intraepidermal melanocytic proliferation were observed in proximity of the follicular openings. Histopathologic examination confirmed the diagnosis of lentigo maligna. The lesion was stable over 5 years with dermoscopy. Dermoscopic images of 2001 and 2003, as well as the most recent one, lacked of specific and diagnostic signs of lentigo maligna, and did not show dermoscopic changes suggestive of a progression growth during the follow-up period. In our case, confocal microscopy allowed to make the correct diagnosis in absence of clinical and dermoscopic suspicion.

A13. In vivo microscopic features of nodular melanomas: dermoscopy, confocal microscopy and histopathologic correlates

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Background: Reflectance-mode confocal microscopy (RCM) is a new tool for the in vivo study of skin lesions. We aimed to characterize NMs by dermoscopy, RCM and hstopatlology. **Methods**: 10 NMs, 10 SSMs with a nodular area (NodSSM), and 10 SSMs with blue-palpable area (BlueSSM) were included. Dermoscopic, confocal and histopathological features were evaluated and a statistical analysis performed. **Results**: Whereas NMs had nonspecific dermoscopic patterns, SSMs exhibited a multicomponent pattern. Atypical vessels, structureless areas, blue-whitish veil, and globules were frequent in NMs and in nodular areas from SSMs. By RCM, NMs exhibited few pagetoid cells within a typical epidermal architecture in the superficial layers, whereas SSMs showed epidermal disarrangement and pagetoid infiltration. At the dermal-epidermal junction, dermal papillae were rarely seen in NMs and in nodular areas from SSMs, substituted by non-aggregated atypical cells. In the upper dermis, all groups exhibited dishomogeneous cell clusters, atypical nucleated cells and plump bright cells. NMs presented characteristic cerebriform clusters. **Conclusion**: Distinctive dermoscopic and confocal features were seen in pure NMs.

A14. Melanomas treated with criotherapy: Mistakes that can be avoided

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Melanoma may represent a diagnostic challenge because it can be a clinical simulator of other skin conditions including tumoral and non tumoral diseases. If melanoma is misdiagnosed inadequate treatments such as criotherapy when applied could make difficult the recognition of melanoma even by dermoscopy

Objectives: to describe the clinical and dermoscopic findings of melanomas treated with criotherapy.

Methods: descrition of a series of six patients referred to the Melanoma Unit of the Hospital Clinic of Barcelona that had been previously misdiagnosed and treated with criotherapy in other centers by chiropodists, general practioners or dermatologists under the diagnostic suspicion of viral warts, basal-cell carcinoma and seborreic keratoses. Dermoscopic description by pattern analyses and histopathologic correlation was preformed.

Results: Criteria of melanocytic lesion and melanoma were observed in all the cases. Due to the previous treatments, the tumors showed significant modification of the dermoscopic structures. Presence of inflammatory changes and regression structures was a main finding in these cases.The histological study of the lesions confirmed the diagnosis of invasive melanoma in all the cases, with Breslow's values ranging from 0,8 to 4 millimeters. During the follow-up, one patient died of metastasic disease.

Conclusions: dermoscopy represents a basic tool in the evaluation of pigmented lesions that avoids a delay in the diagnosis and the application of incorrect treatments in melanoma. Melanomas treated with criotherapy may show significant modifications in clinical and dermoscopy findings that can make difficult the diagnosis of melanoma.

A15. Hispanic Albino with Stage IIIC Melanoma

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33 year old hispanic, albino male was diagnosed with a right leg plaque in 2000. Pathologic diagnosis was 0.45 mm melanoma with extensive regression. A wide and deep surgical resection was done with clean 1 cm margins. He has also had several basal cell skin cancers removed in the past. In October 2004, he developed swelling of the right groin. A fine needle aspiration documented recurrent melanoma. A CT/PET scan demonstrated a 6.5 cm × 6 cm right inguinal mass with no evidence of distant metastatic disease. A surgical resection of right inguinal area was done and an 8 cm × 6 cm × 6 cm right matted inguinal nodal mass replaced by melanoma was removed. 3 additional superficial nodes and Cloquet's node were negative. He was AJCC Stage IIIC (T1b, N3, M0). He refused interferon therapy and did not qualify for HLA-restricted vaccine trials. He was treated with adjuvant temozolomide for 6 months (1/05-6/05). To date, he remains without evidence of disease (last scans done 5/07). He and his wife had their first child 21 months after completing treatment.

A16. Sinus Melanoma in a Patient with Xeroderma Pigmentosum

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18 year old Greek female with Xeroderma pigmentosum who had several resected Stage I melanomas and basal cell cancers removed in the past, developed proptosis, diplopia and numbness in the distribution of the second division of the fifth nerve in October 2006. An MRI revealed a large mass involving the right maxillary sinus, inferior orbit and extension into the cavernous sinus. An endonasal biopsy demonstrated malignant melanoma. An extensive base of the skull and floor of the orbit resection with reconstruction was performed January 2007 and a post-surgical MRI demonstrated a 6 mm focus of residual tumor in the cavernous sinus. This was treated with stereotactic radiosurgery. AJCC maxillary sinus tumor Stage T4b (tumor invades orbital apex and cranial nerve), Nx, M0. Post-operatively, she has completed 2 cycles of adjuvant temozolomide (6 weeks on, 2 weeks off). A recent sinus MRI (May 2007) documented no evidence of disease. She will complete a third cycle of temozolomide and undergo re-imaging. If she remains disease-free, she will be examined every 2 months and imaged every 6 months with a sinus MRI and whole body CT scans.

A17. Lentigo maligna melanoma treated with Imiquimod

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The capacity of Imiquimod to act against tumour cell via activating innate and acquired immunity has been demonstrated. Although the indications accepted are only superficial basal cell carcinomas and actinic keratoses it could be reasonable to use it in special cases. Lentigo Maligna Melanoma normally affecting quite a big area in the face and usually in elderly people with con concomitant diseases can contraindicate the surgical intervention or at least really difficult the procedure. Several cases have been published of the possible usefulness of Imiquimod treating LM. We present a case with a well-established diagnosis of LMM treated with Im, needing several months to obtain the inflammatory response but after that a nice cosmetic result and a histopathologic confirmation of the tumour resolution. A long year and half follow up shows a clean skin.

A18. Case Report: Malignant Melanoma Metastatic of the gastrointestinal tract

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We report a case of recurrent of malignant melanoma in the gastrointestinal tract presenting as iron deficiency anaemia, 14 years after radical surgery for the primary lesion

Case report: A seventy six year old lady was referred to gastroenterology clinic for investigation of iron deficiency anaemia. Her past medical history included malignant melanoma of the vulva excised 14 years ago. She had remained well since then with no evidence of any recurrence. Endoscopy showed focally irregular gastric mucosa in the body of stomach and lesser curvature. Biopsies confirmed the presence of tumour cells that were reactive with melanoma markers-S100, HMB45 and Melanin-A. In the context of previous history, the appearances were regarded as those of metastatic malignant melanoma. Contrast enhanced computed tomography (CECT) revealed both mucosal and sub mucosal metastases to the body of the stomach (fig 1). Associated small bowel mural metastasis causing intussusception was also noted (fig 2). However apart from being iron deficient, she remained well and was referred to the regional oncology centre for chemotherapy. She is currently undergoing chemotherapy on an outpatient basis. CT pictures,discussion & references available for presentation.

A19. An unusual case of a "malignant eczema-like plaque"

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In September 2006, a patient was first referred to our dermato-oncology unit for the excision of a malignant melanoma of the chin (level III, 0.96 mm). In the first examination we discovered another malignant melanoma of the trunk (level II, 0.54 mm). A sentinel node biopsy in the axilla was negative. Apart from these two melanomas, physical examinations revealed an unclear 8 cm × 5 cm large, erythematous plaque on the left upper arm, which presented with an increasing size since 4 years. We first thought of a common eczema or a superficial mycosis, a cutaneous T-cell-lymphoma (CTCL) was considered, too. Surprisingly, histopathology showed typical features of an amelanotic malignant melanoma. The diagnosis was confirmed by three further punch biopsies (Clark-Level III, maximum tumor thickness 0.6 mm). The lesion was completely excised with 1 cm safety side. The wound defect was subsequently closed with a mesh graft from the right tight. Amelanotic malignant melanomas may appear as relatively unsuspicious erythematous maculae or more rarely as plaques. In this unusual case only the biopsy could determine the etiology of this eczema-like malignant melanoma.

A20. Merkel cell carcinoma of the scalp with nodal involvement

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A 74 year old Caucasian male with a long standing history of small lymphocytic lymphoma (SLL) never requiring any treatment, developed a firm nodule on the left, lateral aspect of his scalp. A biopsy originally was read as a glomus tumor, so the patient was followed by his dermatologist with no intervention. Over the course of three months, he developed multiple, ulcerated scalp nodules and an enlarged pre-auricular node. A repeat biopsy was done which was now read as Merkel Cell Carcinoma. The patient was referred to our Cancer Center and a fine needle aspiration of the firm, pre-auricular node was consistent with metastatic Merkel Cell Cancer. A biopsy of several soft left-sided neck nodes confirmed SLL. Original pathology was reviewed and was consistent with Merkel Cell. A CT/PET demonstrated low SUV uptake in the areas of SLL and more intense uptake in the Merkel Cell involved areas. The patient was promptly administered IV etoposide and carboplatin chemotherapy. He demonstrated a prompt response and has had complete clinical resolution of this disease following 6 cycles of chemotherapy. A follow-up CT/PET is scheduled. We plan to consolidate with radiation therapy to the scalp and left-sided nodal area.

A21 was withdrawn during production process.

A22. Synchronic collision tumors: basal cell carcinoma and atypical nevus

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The term Collision Tumor is referred to the association of two distinct neoplasias in the same lesion. This is an uncommon event and a clinical challenge to the practitioner. Accurate diagnosis is important, especially when it occurs between a benign and a malign tumor. Identification of these lesions with dermoscopy has been of great help in this set of patients. We report the case of two synchronic collision tumors diagnosed during a routine follow-up. A 30-year-old female patient with complaint of multiple nevi had previously resected three facial BCC. Eighty melanocytic lesions were identified during naked-eye examination; most of them were clinically dysplastic nevi, therefore characterizing Atypical Nevus Syndrome. Total Body Photography with digital monitoring of the lesions was performed. Three lesions were considered suspicious and resected. Two of them were diagnosed as a collision tumor between superficial BCC's and Atypical Nevi. The surgical margins were free of tumor. The patient remains in short-term follow-up with digital dermoscopy and has no evidence of disease.

A23. Cutaneous melanoma arising from common, congenital and atypical (dysplastic) agminated melanocytic nevi

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The agminated nevus is an entity characterized by grouped melanocytic lesions circumscribed and confined to a localized area of the body. Otherwise of spilus nevus, the main differential diagnosis, the agminated nevus lacks the clinically visible background pigmentation and commonly appears during puberty. Pigmented lesions that have been described as agminated include common melanocytic nevi, congenital melanocytic nevi, Spitz nevi, nevi spilus, blue nevi and multiple lentigines. The report of atypical nevi and malignant transformation within those lesions is rare. Previous reports have documented the existence of common and dysplasic melanocytic nevi arranged in an agminated pattern, however, the occurrence of cutaneous melanoma within this lesion has not been described. We present a case report and dermoscopic features of a 32-year-old white male with atypical nevi syndrome, treated previously for a invasive cutaneous melanoma on the right thigh who develops in his chest an in situ cutaneous melanoma within a common, congenital and atypical agminated nevi with uncommon phylloid pattern.

A24. Intraoperative pencil marking mimicking in-transit metastasis in malignant melanoma

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A 57-year-old woman was recently diagnosed with malignant melanoma on her lower leg, Breslow index 0,61 mm, Clark level II. Excision was performed with a 5 mm safety margin and full-thickness skin was transplanted from her right groin to her lower leg. Two weeks after the transplant she observed six 2 mm measuring black macules around the inguinal surgery scar, which raised the suspicion of intransit metastasis. A skin biopsy was taken for histological workup. Subepidermally, an intra- and extracellular greyish black pigment was surrounded by a lymphocytic infiltrate. No melanocytic nests were seen, and the pigment-loaded cells were negative for S-100, and likewise staining with Melan-A and HMB-45 remained negative. Thus, a melanocytic tumor or melanoma metastasis could be excluded. The pigment was negative for Prussian blue (hemosiderin pigment). Furthermore, the pigment loaded cells did not reveal characteristics of melanophages. In conclusion, human intrinsic pigments could be ruled out. Corresponding to the surgery protocol the size of the skin graft was marked with a blue pencil. In the next two postoperative weeks the extrinsic pigment rests became visible on the site were the skin graft was removed mimicking cutaneous metastasis of malignant melanoma.

A25. Clinical significance of cutaneous melanoma's precursors

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About 75 to 80% of cutaneous melanomas originate on healthy skin, therefore only 20 to 25% of melanomas are thought to develop on a cutaneous lesion that we can identify as a clinical precursor. The main aim should be to clarify the clinical impact of the so-called melanoma precursor and, above all, an attempt to understand which clinical behaviour could be more appropriate for each group. In giant congenital melanocytic nevi, the melanoma's risk has been estimated between 5% and 20%. On the contrary, in small congenital nevi the risk for melanoma seems to be extremely low, therefore the prophylactic exeresis would not be immediately indicated. A great number of Clark's nevi constitute a risk factor for cutaneous melanoma in caucasian patients. The clinical expression of many Clark's nevi could simply be one of the aspects of the patient phenotype, whose skin has a greater relative risk for melanoma, a genetically encoded risk jointly enhanced by environmental factors. It is very important to distinguish the familial dysplastic nevus syndrome, which is a strong risk factor for cutaneous melanoma, from not familial (sporadic) dysplastic nevus, in which the risk for melanoma would depend on the total number of melanocytic nevi, on the phototype and on the relationship to environmental factors.

A26. Prospective evaluation of a four-year period of application of a follow-up protocol for melanoma patient management

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In 2000 the Emilia-Romagna Melanoma Group agreed upon guidelines for the follow-up of melanoma patients, considering histological thickness and disease stage.

Objectives: the purpose of this study was the prospective evaluation of the follow-up protocol after four years, in order to verify the efficacy in identifying resectable metastases.

Methods: during a four-year period, 176 melanoma patients were supervised according to our follow-up protocol.

Results: 39 patients underwent disease progression, showing in transit metastases in 15 cases, lymph node metastases in 11 cases and distant metastases in 13 cases. At the end of the study 11 patients dead for the disease progression. Disease progression was correlated with melanoma thickness, tumor stage and presence of ulceration, with a significant proportion of visceral metastasis in ulcerated melanoma. A low rate of metastases was observed for melanoma of the upper limbs, chest and abdomen.

Conclusions: this study represents a critical intermediate analysis of the efficacy of the Emilia-Romagna protocol after a 4- year of its application.

A27. Melanoma surveillance: an analysis of the early detection of recurrences

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A follow up strategy with reduced imaging techniques in melanoma ≤ 1.0 mm, performed at the department of dermatology in Tuebingen (arm A) was compared to the previous more extended follow up schedule at the departments of dermatology in Kiel and Mannheim (arm B). Follow up examinations were evaluated in stage I-III melanoma patients for a time period of two years. The early detection of recurrences with possible R0 resection was the primary endpoint of the study. Of 3.833 patients 211 presented with 452 recurrences. A reduction of clinical and technical examinations in stage I melanoma (≤ 1.0 mm) did not have an effect on the early detection of recurrences. Loco-regional recurrences were mainly detected by physical examinations and lymph node ultrasound and were discovered earlier in arm A ($P = 0.01$). No difference was observed in the detection of distant metastases, where CT-scans and S100 blood-tests had the highest diagnostic impact. A reduction of physical and technical examinations in primary melanoma ≤ 1.0 mm, application of lymph node ultrasound in melanoma > 1.0 mm and an intensified follow up program in stage III may be considered as appropriate follow-up strategy.

A28. Predicting metastasis using sentinel lymph node biopsy and the shields index

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Sentinel lymph node biopsy (SLNB) is widely used to determine metastatic status. However, this is a highly invasive procedure, does

not affect outcome and 20–30% of patients who are negative at time of biopsy, as determined by histological evaluation, still develop distant metastasis within 10 years (Takeuchi et al J Clin Oncol 2004). In 2004, Shields et al. described a new prognostic index, based on analysis of the primary tumour, including lymphatic vessel density, lymphatic vessel invasion and Breslow thickness (Shields et al Br J Cancer, 2004). In this study we determined the predictive value of the Shields Index within a cohort of 18 patients (8 node negative and 10 node positive) who had previously undergone SLNB. Sentinel node status could be predicted with 89% accuracy by histological assessment of the primary tumour with the Shields Index.

A29. Correlation study between the status of sentinel lymph node(s) and the clinicopathological data of primary melanoma

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Between January 1998 and May 2007, 399 melanoma patients were subjected to a sentinel lymph node (SLN) biopsy in our hospital. Histopathological analysis of the SLN with hematoxylin/eosin and immunohistochemistry was performed in 153 patients. Metastatic SLN were found in 21 patients (13,7%). We have performed a comparative study between the clinical characteristics and the pathological melanoma data of patients with positive and negative SLN. The following parameters were evaluated: age, sex, location, histopathological type, Breslow thickness, Clark level, ulceration, regression, presence and arrangement of the lymphocytic infiltrate, satellitosis, cell type and embolization. Univariate statistical analysis showed that deeper Breslow thickness, nodular type of melanoma, presence of ulceration, absence of regression and absence of lymphocytic infiltrate were more frequently related with a positive SLN.

A30. Predictive value of clinical and histopathological characteristics of primary cutaneous melanoma on the positivity of sentinel lymph node for melanoma micrometastasis

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One of the most important prognostic factors of cutaneous melanoma (CM) is the lymph node status. Sentinel lymph node (SLN) biopsy is a standard procedure in the staging of CM, however its indication is based exclusively on some histological data of the primary tumor. The objective of this study was to correlate clinical and histopathological features of the primary tumor with the positivity of SLN for micrometastatic melanoma and possibly identify risk groups for metastatic disease. Two groups of patients: with and without metastatic disease at the SLN were compared. From September 1997 to December 2003, 265 patients where enrolled, fifty-four in the positive sentinel group and 211 in the negative sentinel group, with minimum follow-up of 2 years. Univariate and multivariate analysis of skin fototype, familial history, clinical ulceration of primary tumor, medium or giant congenital melanocytic nevi, melanoma subtype, Breslow thickness, Clark's level, ulceration, mitotic index, vascular, lymphatic and perineural invasion, regression and satellite nodules were performed.

A31. Melanoma in a general surgery department

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General Surgery Department(1) Dermatology Department(2) Surgical Pathology (3) Nuclear Medicine (4) Radiotherapy (5) Oncology (6) Pharmacy (7) Internal Medicine (8) .CHOU Ramón Puga st, 52–54. CP: 32005 Ourense, Spain

The principal objective is the description of the surgical treatment in the melanoma in our General Surgery Department. Since 2000, the Complexo Hospitalario de Ourense created a multidisciplinary unit in the General Surgery Service specialized in melanoma treatment. This unit elaborated a protocol integrating several medical specialities and new aspects in the melanoma treatment that had a positive impact in the management of our patients and the experience of the medical team. Being this center a referral hospital in the region this permited to create a guideline for the patients management. Our unit has established methodology to avoid unnecessary waits or moves, creating an ambulatory surgery unit. A Sentinel Lymph Node Biopsy program started up and at he present time 115 patients have been operated with only one false negative. The surgical experience in microsurgery has allowed us skin coverages using pediculate and perforate grafts. Principally, the metastasis treatment has been developed with linphadenectomies (axillary, inguinal and cervical regions), ongoing metastasis and hepatectomies. Our unit has a specific educational program for resident interested in melanoma management. Conclusions: Our unit is a representative model of a multidisciplinary group for the treatment of melanoma.

A32. Regional lymphadenectomy in melanoma treatment

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Objectives: a systematic review of all Regional Lymph Node dissection in melanoma in a surgical unit

Methods: we reviewed 27 cases treated by the same surgeon in our Unit. The principal reason for the node dissection was a positive sentinel node or clinical nodal metastasis. The Regional Lymph node dissection were located in the following areas: axillary region in 16 patients, inguinal region in 7 patients and cervical zone in 4 patients. Two patients (one from cervical region and other from axillary region) have been treated previously with a bilateral regional lymph nodal dissection. Main surgical complications were serous collection in axillar and inguinal regions, and several degrees of wound necrosis that were observed only in the inguinal region. The surgical technique was radical in all cases, but tried to preserve the nerves in the region. No significant neurological deficit has been noted. One patient with a knee prosthesis showed a prosthesis infection in the post-operative period that required its removal.

Conclusions: the regional lymph node disection continue to be a difficult-to-manage surgery, principally in inguinal region. Significant lymphatic damage was not observed in cases with sentinel lymph node metastasis. Because of the importance of standardisation of the procedures, these surgical treatments are better establish in referral centers.

A33. Utility of sentinel lymph node biopsy in melanoma

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Sentinel Lymph Node Biopsy is an accepted method for breast cancer but in melanoma persists controversy about its real utility. The variability of the method gives hetereogeneous results with false negatives and surgical morbidity, especially in the inguinal region. In the main positive biopsies, only one sentinel lymph node is affected. In addition to this the lymphogammagraphic study permits a good preoperative evaluation of all the drainage tracts. When sentinel lymph node anatomopathologic study is exhaustive this is the main prognostic factor

that assists the precise staging of the patient. In addition to this sentinel lymph node biopsy and gammagraphy identify aberrant drainage zones.

In this study the results of 115 sentinel lymph nodes biopsies performed by an experienced team are reviewed retrospectively. From 115 patients all sentinel lymph node have been detected, except two cases. Two cases were considered false positives. Several cases of aberrant circulation in the so-called interval zones, principally in supraclavicular and popliteal regions were observed. A correlation was identified between progression to distant metastasis and survival and status of the sentinel lymph node.

Conclusions: Sentinel Lymph Node Biopsy is a useful technique for the accurate staging of the patients with prognostic value.

A34. Melanoma and integra

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New concepts of the treatment of melanoma have been incorporated in our Surgical Unit of Melanoma. One of these treatments is the reconstruction with synthetic or artificial dermis. Artificial dermis has been used successfully for full-thickness coverage wounds with a well-vascularised surgical bed. We present a patient with a superficial spreading malignant melanoma (Breslow thickness 3,2 mm on the leg. After local surgery and sentinel lymph node biopsy, the defect was repaired with a skin substitute (Integra). A split-thickness skin graft (0.011 +/– 0.0 inch) was placed on the operative site at 36th post-operative day after removal the silicone layer of the artificial dermis. Clinically, the excision area reconstructed, showed well-vascularised neodermis before skin grafting. There was skin graft with no infection or other complications. The reconstructive procedure was performed in less than 1 hour of combined operative time, with the last stage performed over an outpatient basis. Conclusions: Artificial dermis can be used successfully for reconstruction of defects following oncologic resection, offering minimal donor-site morbidity and expedient operative time. Integra skin may offer another option for definitive management of extensive full-thickness defects.

A35. Molecular Nodal Staging of Pathologically Negative Sentinel Nodes from Melanoma Patients using multimarker quantitative real time PCR

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Pathological exam of SLN provides crutial prognostic information in melanoma patients, but its sensitivity is suboptimal. We hypothesized that occult metastases can be a source of recurrences in pathologically negative SLNs.

Methods: Patients with AJCC stages I-II melanoma who underwent SLN biopsy (1997 to 2004) and without evidence of metastases after pathological exam were included. RNA was isolated from the frozen half SLN. The expression of the melanoma-associated mRNAs MART-1, Tyrosinase and MAGE-A3 was evaluated through quantitative RTPCR. The results were correlated to disease evolution. Results: 370 dissected SLN from 195 patients (male/female: 67/128) were obtained. The mean Breslow thickness was 1,7 mm (18% ulcerated), and 11 of the 195 patients recurred in a median follow-up period of 49 months. We got 36% of the patients positive for one or more markers (31% positive for Tyrosinase, 24% for MART-1 and 10% for MAGE-A3). Kaplan-Meier method did not show significant differences in survival based on individual markers or marker combinations.

Conclusions: In our study, quantitative multimarker RT-PCR failed to identify patients with higher risk for recurrence. However, differences between groups may have not reached statistical significance due to the scarce number of recurrences in our set of patients. On the other hand, recurrences in the pathological negative SLNs patients may have originated in sources other than occult metastases in SLN.

A36. Dermoscopic changes in treatment of displastic nevi with 5% imiquimod cream

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The management of patients with melanoma associated with multiple atypical moles (Displastic nevi syndrome), that are equivocal respect to their benignity by clinical examination and dermoscopically, is disputed. The surgical treatment of all those lesions is at present in dispute, mainly when most of them correspond to a benign dysplastic nevi. We describe the case of 3 patients with atypical mole syndrome treated with 5% imiquimod cream, according to the classical guideline of a daily application 5 days per week. We followed-up the atypical melanocytic lesions by clinical and dermoscopical monitoring during several months to evaluate the evolution of those lesions treated with topical inmunomodulador. This study suggests a possible role of the chemoprevention with imiquimod in atypical melanocytic lesions and their role in the prevention of their progression to melanomas.

A37. Markers of cutaneous malignant melanoma in peripheral blood

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Markers of Cutaneous Malignant Melanoma in Peripheral Blood Ziman M.1, Medic S.1, Slattery R.1, Pearce R.1, Heenan P.2 1 Edith Cowan University, Perth, Australia 2 University of Western Australia, Perth, Australia In situ melanoma is largely curable by surgical excision of the lesion with a 95% survival rate. In contrast, the five year survival rate is drastically decreased (by $>40\%$) in patients with advanced metastatic disease. Finding sensitive markers to detect dissemination is a major research focus as metastasis may be undetected clinically and recurrence can occur many years after surgery. We have used RT-PCR and Realtime RT-PCR to assess the presence of several genetic markers of circulating melanoma cells in peripheral blood from 125 melanoma patients and 40 healthy volunteers. The frequency and level of expression of markers was correlated to Breslow tumour thickness and results were statistically analysed. Results show that markers of circulating melanoma cells were only detected in blood of melanoma patients and not in samples from healthy volunteers. One marker in particular showed a higher detection rate overall, regardless of tumour thickness, although levels were highest in those patients with thicker tumours. Further research is progressing to increase the sensitivity of detection and characterise the migrating cells.

A38. Analysis of the multiple serum markers in advanced MM

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Background: Serum levels of YKL-40 have recently been demonstrated to be an independent prognostic factor in melanoma. The purpose of this study was to analyze the expression of YKL-40 in advanced melanoma patients and its correlation with other known

melanoma markers including S-100β, melanoma inhibiting activity protein (MIA), and lactate dehydrogenase (LDH). **Methods**: Serum YKL-40, S-100β, MIA, and LDH levels were measured in fifty three advanced melanoma patients and ten healthy controls. **Results**: Serum levels of these markers were significantly higher in melanoma patients than in healthy controls. YKL-40 sensitivity (92%) was higher than S-100β (74%), MIA (64%), and LDH (72%). Moreover, the combination of YKL-40 and S-100β (96%), MIA (94%), or LDH (98%) showed higher sensitivity than any single marker. The AUC (area under curve) for YKL-40 was 0.97, which was significantly higher than for the other melanoma markers by Receiver Operating Characteristics (ROC) analysis (S-100β, AUC = 0.81; MIA, AUC = 0.71; and LDH, AUC = 0.85). The number of positive serum markers was a prognostic factor for survival. Patients with four markers had a median overall survival of 3.7 months versus 11.23 months in those with less than four markers ($p < 0.001$), and this was confirmed as an independent prognostic factor by Cox regression analysis (HR = 3.56, $p = 0.024$). **Conclusions**: YKL-40 showed better sensitivity and diagnostic accuracy in melanoma than the other markers explored (MIA, S-100β, and LDH). The analysis of multiple serum markers in metastatic melanoma is also of prognostic relevance.

A39. Quantitative RT-PCR (qRT) analysis of melanoma sentinel lymph nodes (SLNs): prognostic significance of a multimarker molecular assay

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Purpose: To study the prognosis prediction by determining multiple melanoma markers in melanoma SLNs by quantitative real time reverse transcriptase polymerase chain reaction (qRT) assay. **Patients and Methods**: One hundred and fifty seven melanoma patients who underwent SLN biopsy were included. A portion of each SLN was stored frozen at –80°C and assessed by qRT for mRNA of three genes: MART-1 (antigen recognized by T cells-1), MAGE-A3 (melanoma antigen gene-A3 family) and tyrosinase. The remaining node tissue was analyzed by serial section histopathology technique. **Results**: Twenty-five (15.9%) patients had histopathology-positive SLNs. The percentage of patients with positive results for tyrosinase, MART-1 and MAGE-A3 in histopathology-positive and negative subsets were as follows: 88%, 68%, 32% and 56.06%, 29.54%, 6.8%. Although single markers and their possible combinations shown prognostic values in the univariated analysis for disease free survival, only the simultaneous expression of tyrosinase and MART-1 (51 patients) had an independent prognostic value (HR:2.72; 95% CI, 1.21-6.13; $p = 0.016$). **Conclusion**: These findings suggest that the simultaneous expression of MART-1 and tyrosinase mRNA in melanoma SLNs identifies a subgroup of early stage melanoma patients with an increased risk of recurrence.

A40. Whole brain radiotherapy following local treatment of 1–3 intracranial metastases of melanoma – a phase III trial

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Whole brain radiotherapy (WBRT) following local treatment of intracranial melanoma metastases is controversial. Proponents say prolongation of intracranial control reduces neurological events and provides better palliation. Opponents say WBRT gives no survival benefit and may impair neuro-cognitive function. These opinions are based on other tumour types that may have different radiation sensitivity. A randomised controlled trial is proposed. Previous WBRT trials in other malignancies in Australia have accrued poorly due to bias of individual clinicians against WBRT. We hope to interest melanoma units from around the world, as the multidisciplinary nature and openness to evidence-based medicine of these units will help achieve the target accrual. A feasibility study of 29 centres has been performed showing that international collaboration would be advantageous. Choice of quality of life and neurotoxicity instruments is controversial and will be discussed.

A41. Radiotherapy and melanoma – a single institution experience

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Introduction: Radiotherapy is not widely used in the management of metastatic melanoma. It does have an important role in palliating symptoms. The aim of this review was to determine the demographics, common sites of radiotherapy treatment, benefits and survival of metastatic melanoma patients treated with radiotherapy at our institution. **Methods**: Radiotherapy records were searched for patients who received palliative radiotherapy for malignant melanoma between 2001 and 2007. Dose, fractionation and site of radiotherapy were recorded. Patient demographics, response and survival data were obtained. **Results**: 84 patients were identified; mean age 57 (range 21–89), M:F ratio was 2:1. 90% of patients were deceased at the time of analysis. 54 patients (64%) received radiotherapy for cerebral metastases. In 49 patients sufficient data was available; median survival; 4 months overall and 10 months in those receiving radiotherapy after neurosurgical excision; 39% survived more than 6 months. 23 patients received radiotherapy for bone metastases, 10 had follow up after radiotherapy, 9 of whom had clinical benefit. 8 had soft tissue lesions, 5 were followed up after radiotherapy and 4 had clinical benefit. **Conclusions**: This review strongly supports the use of palliative radiotherapy in melanoma patients.

A42. Treatment of cutaneous metastasic malignant melanoma with intralesional IL-2

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Melanoma is the cutaneous malignancy with the highest mortality, and the incidence of melanoma continues to increase worldwide. Patients with advanced malignant melanoma can be difficult to treat effectively. In these patients several treatments had been evaluated with variable results. The host immune system plays an important role in the defence against malignant melanoma, that's why in the last decade interest has been focused on immunotherapies. IL-2 is an immunotherapeutic agent that clinically has been evaluated in several doses and schedules. Intralesional IL-2 was found to be highly effective for the treatment of skin or soft-tissue melanoma metastasis in a phase II clinical trial. We report a case of multiples cutaneous metastasis of malignant melanoma with good local response to treatment with intralesional IL-2.

A43. Adoptive transfer of autologous tumor-infiltrating-lymphocytes for treatment of metastatic melanoma patients

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Purpose: Melanoma is an immunogenic tumor infiltrated by T-lymphocytes, which may have the potential to destroy the cancer cells. SA Rosenberg reported a study, in which selected autologous tumor-infiltrating-lymphocytes were administered to lympho-depleted metastatic melanoma patients. 51% (18/35) objective response rate, including three complete responders, was achieved. The "Ella-Institute" adopted this technology and provides here the preliminary

results. **Clinical Study**: Eligibility criteria: Stage IV melanoma patients, pre-treated with chemotherapy and IL-2, good performance status, no brain metastasis. **Clinical Protocol**: Non-myeloablative lympho-depleting chemotherapy with cyclophosphamide and fludarabine prior to TIL infusion followed by bolus high-dose IL-2. **Conclusions**: Until today twenty-three patients entered our protocol. Six completed therapy, whereas fourteen patients did not receive TIL infusion, due to lack of anti-tumor reactive TIL or development of brain metastasis during TIL preparation. Three patients are still in the preparatory phase. Five treated patients developed neutropenic fever, which was managed successfully with antibiotics. No treatment related death or major toxicity (grade 3/4-beside from neutropenic fewer and expected pancytopenia) were observed. Two out of six patients achieved a partial remission. The preliminary data shows, that this technology is feasible and encouraging.

A44. Potent anti melanoma immune response followed by adoptive transfer of T helper 2 profile CD4+ cells

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The antitumoral activity of CD8+ cytotoxic T-lymphocytes (CTL) has been widely demonstrated. T helper (Th) 1 type immune response enables the required activation of both CTLs and NK cells. On the other hand, Th2 type response was proved to provide a suppressive environment for immune responses and was even documented as a central immune evasion strategy taken by tumors in order to create an anti inflammatory tumor environment. We describe here a stage IV melanoma patient who underwent adoptive transfer of tumor-infiltrating-lymphocytes (TIL) and experienced a major partial response. CT scan performed four weeks post transfer revealed a dramatic response at all lesion sites. Retrospect examination of the TIL properties revealed a typical CD4+ Th2 profile (>98%). This TIL was characterized by homogeny Th2 antigenic phenotype, secretion of Th2 cytokines, lack of granzyme B or perforin and no cytotoxic effect towards autologous melanoma cells in vitro. The clinical response of this patient might raise new aspects that have not been taken under consideration so far in the mechanism mediating the anti-tumor immune response.

A45. Combination of therapeutic vaccination with autologous mRNA electroporated dendritic cells and interferon alfa-2b results in skin depigmentation and tumor regression in patients with advanced melanoma

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We recruited 28 pts with stage IIIC-IV melanoma. Following leukapheresis and enrichment in a semi-closed culture system, adherent PBMC are cultured in IL-4 and GM-CSF supplemented medium for 6 days and cryo-preserved. Upon thawing, 15 miljon DC are electroporated with synthetic mRNA that encodes a fusion protein between LAMP and one of 6 melanoma associated antigens. DC vaccines are administered by 6 bi-weekly ID/SC injections and q8 wks thereafter. The first cohort received DC-vaccination alone and IFN-α2b at progression. The second cohort initiated DC-vaccination and IFN-α2b (5 miljon IU sc 3x/w) together. Two pts experienced regression of subcutaneous metastases during DC vaccination and 1 of these pts remains disease-free after 24 months of follow-up. Three out of 4 pts in the first cohort who were treated with IFN-α2b at progression developed skin depigmentation. In 2 of these pts an objective tumor response was documented (including complete regression of visceral and skeletal metastases). Both responses are ongoing at 16 and 24 months of follow-up. In cohort 2, 4 out of 11 evaluable pts have experienced skin depigmentation.

A46. Massive regression during treatment with Ticilimumab in selected stage IV melanoma patients

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Ticilimumab is a new therapeutic monoclonal antibody directed against human CTLA4, which is currently investigated in a large phase III clinical trial in advanced melanoma patients. Treatment is applied intravenously every 3 month at a dosage of 15 mg/kg and aims to overcome tolerance to melanoma cells by inhibiting regulatory T cells. Here we report two selected patients with massive regression within few weeks after start of therapy. Patient 1 initally showed M1c disease with hepatic and pulmonal metastasis which regressed dramatically even after 6 weeks of treatment. In patient 2 a complete response of pulmonary metastasis could be demonstrated after one treatment cycle. These regressions were not accompanied by any clinically significant toxicity. Until now both patients are still receiving treatment almost 12 month after their initial cyle due to their sustained responses.

A47. Endoplasmic stress (ER) in melanoma cells

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Endoplasmic Reticulum Stress Drives Malignant Progression of Melanoma . The most powerful predictor of prognosis of metastatic melanoma is LDH levels. LDH is needed for anaerobic glycolysis and elevation of serum levels is an indicator of the metabolic need of the melanoma cells and their need for glucose. The hypoglycemic state also triggers endoplasmic reticulum (ER) stress pathways. In normal cells this causes cell death but melanoma cells have adapted to ER stress by upregulation of survival pathways. One of these is the MEK/ERK pathway. We show here how induction of ER stress may upregulate TRAIL death receptors but also induce resistance to chemotherapy by mechanisms involving glucose regulated protein GRP78. These insights promise to provide new treatment initiatives in highly malignant glucose avid melanoma.

A48. A combined morphometric and ribonucleic acid extraction analysis of melanoma samples

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When extracting tissue for RNA in gene expression analysis, heterogeneity in biopsies of melanomas creates a need to know the relative contributions due to tumor versus other cells. Morphometric analysis of sections of specimens can utilize simple point-counting methods to give valuable data about heterogeneity. The combined morphometric and biochemical study of 60 archived metastatic melanoma biopsies showed that proliferating melanoma cells contributed by far most of the total RNA extracted (Qiagen RNA easy method) when the sample was more than 50% tumor. Collagenous tissues, such as scars, and muscle contributed very little to the total RNA. A plot of % tumor vs uG total RNA allowed a selection of a group of samples with >50 % tumor and >90 uG of RNA, which were used as a standard for comparison to the other samples. With Affymetrix Human Genome U133A microarrays, the amounts of microphthalmia-associated transcription factor (MITF) paralelled the plot of total tumor RNA, and the expression of Cyclin D1 usually paralleled the count of mitotic figures on the slides, except in sclerotic melanoma. The diagnostic value of these findings requires future prospective study.

A49. Effect of proteasome inhibitors on proliferation and apoptosis of melanoma cell lines

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Introduction: Proteasome is a promising anticancer target. The effect of 4 Proteasome Inhibitors (PI) on proliferation and apoptosis of melanoma cells was studied. M&M. Sixteen melanoma cell lines were treated with four PI (Bortezomib, MG132, ALLN, epoxomicin). 1) Viability studies: MTT assays. 2) Proliferation studies: BrdU incorporation. 3) Cell death/apoptosis studies: Hoescht and propidium iodide staining; assessment of caspase activation by western blot; evaluation of cell cycle by flow-cytometry. **Results**: After 24–48 h, PI induced a strong viability decrease in 60–80% of cell lines. In sensitive cell lines, PI produced decrease of BrdU incorporation, cell cycle arrest and cell death. Cell death was caused by caspase-independent and -dependent (caspase activation, subG1 peak, nuclei morphology, inhibition by BAF) pathways. Longer PI treatments (6–8 days) decreased viability of apparently PI resistant cell lines. **Discussion and Conclusions**: Our results suggest that proteasome inhibition could have a role on melanoma-targeted therapy inducing cell death by caspase-dependent and -independent mechanisms. Grants: FIS-PI060832 / AECC, Catalunya contra el Càncer, Lleida

A50. Expression of somatostatin receptors (SSTR) in melanoma cell lines. Effect of somatostatin analogues on their proliferation

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Somatostatin analogues (SA) are potential anticancer agents. The expression of SSTR in melanoma cells and the effect of two SA on their proliferation and viability were studied. **M&M**: Nineteen primary and metastatic human melanoma cell lines were treated with two SA (Octreotide and SOM-230). Expression of SSTR1, SSTR2, SSTR3 and SSTR5 was assesed by real-time PCR. Proliferation, viability and apoptosis were assesed by MTT and LDH assays and Hoescht staining.

Results: Most cell lines express one or more SSTR. Both SA (stronger SOM-230) inhibit the proliferation of most cell lines, but never $\geq 50\%$. Cell viability decrease or apoptosis were not detected.

Conclusions: Our results suggest that melanoma cell lines express SSTR. However, the SA investigated (Octreotide and SOM230), at the conditions used in this study, do not significatively inhibit melanoma growth or induce cell death. Supported by NovartisPharma SA and the Research Group of Oncological Pathology of IRBLLEIDA.

A51. Malignant melanoma: what is its situation in Algeria and in the Maghreb. State of this tumor in 2007

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Malignant Melanoma (MM) is a tumor observed in all the races and regions of the world. The lack of consistent data on its frequency in the countries of Maghreb lead us to study its situation starting with some series of survey in the MM registered in Algeria, in the Morocco and in Tunisia.

Methods: the survey consisted in the review of the whole published works or cases of MM presented in the countries of the Maghreb.

Results: 1-Most authors of Maghreb underline the rarity of MM outside of X.P. In Algeria, Boudghene Stambouli et al recovered in 10 years, 10 cases and 16 cases between 1981 and 1995 in Tlemcen. Bouadjar et al, in a national survey (multicentrique) concluded the rarity of this tumor. In Morocco, El Ouazzani et al also noted the rarity (82 cases in 19 years). In Casablanca Benzekri et al, from 4316 cutaneous cancers observed from 1971 to 1991, only 3,5% corresponded to MM. Bennouna Biaz et al also observed the low frequency of MM. Between January 1973 and October 1994 in Tunisia, Gharbi collected 30 cases in 13 years in Tunis and Fazaa et al also described this low incidence. 2-The location in more than 50% was the inferior extremities with a predominance of the plantar region and the heel in the documented cases in Algeria, Morocco and Tunisia. 3-The predominance of nodular MM was observed in Algeria, Tunisia, and Morocco.

Conclusions: MM is a rare tumor in the population of the Maghreb. The constitutional pigmentation observed in the countries can explain the low incidence due to the natural protection against the UV radiation involved in the pathogenesis of MM. The lower extremities and notably the plantar area is the predominant site of MM.

A52. Incidence of different histologic types of melanoma during the period 1996–2006 in the central part of Bosnia (Ze-Do Canton)

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The number of skin malign neoplasms is constantly increasing. Malignant melanoma has shown the greatest increase in its incidence. A retrospective and prospective, targeted, controlled and open study covered the period from 1996–2006 and included all the patients with melanoma verified and histological confirmed by the Department for Pathological Anatomy and Histology of the Cantonal Hospital in Zenica. During the 1996–2006 period 99 melanomas have been detected. MM was found with equal frequency in the males and females. Description of the characteristics of the tumors and patients will be provided.

A53. Differences in cytokine levels in melanoma patients with and without redness (Brenner's sign)

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An observation of malignant melanoma patients who had a reddish appearance that could not be attributed to any medical cause including photosensitivity was reported earlier. Sera from 19 patients with malignant melanoma, out of which 13 had redness and 6 did not, were studied for the expression of IL-6, IL-8 and S-100. The mean level of IL-6 in the group with redness was higher with statistical significance than in the group without redness, and the serum level of IL-8 was insignificant in 7.6% in the redness group, and in 50% in the group without redness, with a statistical significance. The mean level of S-100 in the group with redness was higher than in the group without redness with no statistical significance. The higher plasma levels of IL-6, IL-8, and S-100 in the redness group may reflect a more advanced disease, and serve as an additional prognostic tool, or as a marker of the efficacy of the treatment.

A54. Melanoma risk factors in the elderly

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No studies have focused on melanoma risk factors in elderly population.

Methods: a prospective study was carried out of a case controlled group. From the over 60's assessed between 1997 and 2005 in the Dermatology Department of the Instituto Valenciano de Oncología, (IVO) Valencia, Spain and diagnosed as having malignant melanoma, 160 patients were selected. As a control group, 320 patients assessed during a health promotion scheme in the Elderly carried out by the City Council of Valencia were included. Both groups were assessed for different phenotype characteristics (hair and eye colour, photo type), the presence of other cutaneous lesions (solar freckles, radiation keratosis and nevus), degree and type of solar exposure and personal and family past history of cutaneous, or other, cancer. Contingency tables and logistic regression models were used as evaluation tools.

Results: risk factors of developing malignant melanoma in the Elderly are: tobacco, past history of cutaneous and other cancers, sunburn, photo type, hair and eye colour, number of nevus, solar freckles and solar keratosis. In the multivariate study persistent risk factors were tobacco, past history of cutaneous or other cancers, sunburn, hair colour, the number of nevus and occupational sun exposure.

Conclusions: these results should be taken into account in campaigns for the prevention of malignant melanoma in the elderly.

A55. Different risk associations of melanoma based on anatomical distribution in a Mediterranean patient population

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Recent evidence suggests that cutaneous melanoma arises from diverse causal pathways, one associated with melanocyte proliferation and the other with chronic exposure to sunlight. Melanomas on sun-exposed locations are more likely to develop in people with many actinic keratoses and chronic sun exposure, whereas truncal melanomas more often among individuals with multiple nevi and a history of intermittent sun exposure. We tested this hypothesis in a study comparing clinical characteristics in melanoma patients from a relatively dark-skinned Mediterranean population. Our analysis included 113 melanoma patients, 43 with head and neck melanomas and 70 with truncal melanomas. A clinical evaluation of common nevi, actinic keratoses and solar lentigenes was performed. Chi-square test was used for data evaluation. An association between the presence of nevi or actinic keratoses and the location of the tumor was detected. Patients with truncal melanoma had more often multiple nevi (60.9% vs 21.4%).

A56. Acral lentiginous melanoma is a clinical-pathologic distinct entity

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Acral lentiginous melanoma presents with epidemiologic evidences that suggest it is different from other histogenic types. We aimed to compare clinical, epidemiologic and pathological differences between acral lentiginous melanoma and the rest of more common histogenic types. A series of 978 patients with cutaneous melanoma from Valencia, Spain, were studied. Variables were selected from the database and included data on clinical, histologic and epidemiologic features. Chi-squared test (and Fisher test when appropriate) and ANOVA test. Forty-six (4,7%) patients had ALM type. ALM had statistically significantly thicker tumours, more frequently ulcerated, with less inflammatory infiltrate and less frequently associated to a previous melanocytic lesion. Epidemiologic differences were conspicuous for the older age at diagnosis, lower rates of sunburns, lower number of common and atypical melanocytic nevi and, particularly, a stronger personal and familial history of other malignancies different from melanoma. All these data kept their significance after adjusting for age and sex. Our results contribute to reinforce from a clinical and epidemiologic point of view recent data on genetic characterization of melanomas. In comparison with the other frequent variants we have shown that ALM had some important differences that emphasize that it is a distinct entity more probably related to some cancer susceptibility genes but unrelated to familial melanoma.

A57. Risk factors for cutaneous melanoma: sun exposure, nevi and tanning bed use

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Most epidemiologic studies on risk factors for melanoma have been performed on fair skin populations. Our objective was to identify risk factors for development of melanoma in a Mediterranean population. A case-control study for performed including 293 melanoma patients and 293 age and sex matched controls from the same geographical area. Distribution comparisons for age, sex, family and personal history of cutaneous melanoma, of non melanoma skin cancers and of other neoplasias, sunburns, cutaneous phenotype (phototype, hair and eyes colors, number of common nevus, number of atypical nevus, lentigines presence) and tanning bed use were performed. After adjusted by all the significant variables, the risk of melanoma was strongly related to number of common and atypical nevi, presence of solar lentigines and past history of other non-melanoma skin cancers while UVA lamp beds use was a protective factor. The presence of solar lentigines and the personal history of NMSC as independent risk factors enhances the role of sun-host features interaction and seems to bond the role of excessive sun exposure adjusted by personal sun sensitivity. The importance of a nevogenic pathway is also highlighted with the presence of melanocytic nevi in the multivariate model. The controversial protective role associated to UVA lamp bed use should be confirmed in future studies.

A58. CDKN2A and CDK4 mutation analysis in familial melanoma in Valencia, Spain

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Mutation analysis of *CDKN*2A and *CDK4* genes was undertaken by direct sequencing in 46 probands with a family history of melanoma. A family history of melanoma was considered when there was at least one first or second degree relative with histologically confirmed melanoma. We identified a total of 9 (19.6%) *CDKN2A* germline mutations and 3 cases (6.5%) with the Thr148Ala polymorphism. Of all these 9 mutations 8 mutations were located in exon 2: three Gly101Try, two 358DelG, two Val59Gly and one Asn71Ser; and 1 mutation was located in exon 1 beta: Gly122Arg. Among several phenotypic features, only the number of family members with melanoma (mean: 1.89 vs 1.16, $p = 0.022$) and the presence of multiple melanomas (5/9 vs 5/37, $p = 0.015$) were statistically significantly associated to the presence of *CDKN2A* mutation. A trend to present darker pigmentary traits (higher phototype, dark hair and eyes) and larger number of both atypical and common melanocytic naevi was found among *CDKN2A* mutation carriers. Interestingly, the patient that carried the exon 1 beta mutation presented the higher number of family members with melanoma, and one of the patients with the Gly101Try mutation had also a second degree relative with pacreatic cancer.

A59. Genetic risks factors for melanoma development in Latin-America

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Genetics Melanoma Network (GenoMEL) focused in melanoma (MM) genetic susceptibility (GS) is obtaining data from Latin-America. Objective: To study well-known genes for MMGS (*CDKN2A*/ *p14arf*, *CDK4, MC1R*) in familial and multiple-primary MM in Latin-America.

Methods: Three years period (2006–2009). Patients from Latin-America. Until now: 16 pedigrees (25 individuals) from Mexico and Uruguay.

Results: I49T, M52T *CDKN2*A-mutations identified in Mexico. E88X, -34G > T, G101W in Uruguay. Mutations percentage: 100% in families with 4 MM (1/1 family), 25% in 3 MM (1/4), 55.5% in 2 MM (5/9) and no mutation in MPM.

Conclusion: CDKN2A-mutations responsible for MMGS in Latin-America. Nonsense germline E88X previously reported as somatic change in MM cell lines was detected in two unrelated Uruguayan families. M52T detected in Mexico was not previously described. One MPM Mexican patient was believed homozigote for I49T by sequencing but later MLPA showed deleted exon 1 alfa.

A60. Comparison between familial and sporadic cutaneous melanoma in Valencia, Spain

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Objective: to compare clinical, epidemiologic and pathological differences between familial and sporadic melanoma patients.

Methods: A series of 959 patients with cutaneous melanoma from Valencia, Spain. Variables: age, sex, melanoma site, histogenetic subtype, tumor thickness, stage, family and personal history of cutaneous melanoma, of non melanoma skin cancers and of other neoplasias, sunburns, cutaneous phenotype (phototype, hair and eyes colors, number of common nevus, number of atypical nevus, lentigines presence), presence of lentigines at melanoma site. Chi-squared test (and Fisher test when appropriate) and ANOVA test. Odds ratio were obtained in both univariate and multivariate analysis by logistic regression.

Results: Forty-one (4,28%) familial and 918 sporadic melanoma were identified. A younger age at diagnosis, red/blonde hair, presence of clinically atypical nevi, development of multiple melanomas, and a lower rate of cases with lentigines in melanoma site. Except for hair color and age, the other variables remained statistically significant for their association to the family melanoma after the multivariate study.

Conclusion: phenotypic risk factors for familial melanoma are a tendency to present melanomas earlier in life, to develop multiple melanomas, to have clinically atypical nevi and to present less actinic damage at the melanoma site.

A61. CDKN2A mutation analysis in patients with melanoma and breast cancer

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Carriers of mutations in the melanoma susceptibility gene, CDKN2A, exhibit a higher than expected risk of breast cancer. **Objective**: To determine mutations in the CDKN2A gene in patients with melanoma and additional breast cancer.

Methods: Twenty-seven patients with histologically confirmed melanoma and breast cancer were studied for CDKN2A/ARF gene mutations by direct sequencing analysis.

Results: We identified three CDKN2A germline mutations. A patient with a melanoma diagnosed at the age of 58 and a breast cancer at 62 without family history of cancer harbored the Ala148Thr polymorphism. Another patient with a melanoma diagnosed at 77 and a breast cancer at 66 and a family history of melanoma, had the Val59Gly mutation, a well known mutation that is believed to be derived from a single ancestral founder of Mediterranean (possibly Jewish) origin. The third patient had a melanoma diagnosed at 54 years, a breast cancer at 46, and a strong family history of breast cancer (mother and grandmother), and presented the Ala85Thr mutation.

Conclusions. The epidemiologic link between cutaneous melanoma and breast cancer is not mainly related to CDKN2A mutations. However, some mutations could have a role in this association or even in familial breast cancer, as it could be inferred from the patient with the Ala85Thr mutation.

A62. Genetic predisposition in twelve Spanish mediterranean melanoma-prone families

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We explored the genetic predisposition to develop melanoma in twelve families, with at least two melanoma relatives, from a low incidence region. We also examined in these families, those major features previously associated with the presence of CDKN2A germline mutation: early age-onset (50), pancreatic cancer cases and family members with multiple primary melanomas (MPM). Phenotype variables were assessed by clinical examination and germline mutations were analyzed by direct sequence of MC1R, CDK4 exon2 and CDKN2A. G101W germline mutation of CDKN2A was detected in 25% of families and nonRHC MC1R variants (V122M and V60L) were also identified in five G101W carriers. RHC MC1R variants (R151C, R142H, R160W) were detected in 56% of families without germline mutations in CDKN2A, a high frequency probably related with selection bias. NonRHC MC1R variants were detected in 42% of the families and all of them were heterozygous variants. In our families, the application of features associated with CDKN2A-mutations provided an insufficient positive predictive values for the genetic test selection of patients, suggesting the need to evaluate different combination of them with stringent criteria in higher number of families.

A63. Genetic susceptibility to melanoma

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Introduction: CDKN2A and CDK4 are two major susceptibility genes for melanoma (MM). Other genes involved in melanoma susceptibility are MC1R and genes related with nevogenicity (c9orf14).

Objectives: To describe the prevalence of germ line mutations in CDKN2A and CDK4 in familial melanoma, multiple primary melanomas (MPM) and sporadic melanomas in Spain; to evaluate the modifying effect of MC1R polymorphism, c9orf14 variants and some clinical variants and to describe a specific surveillance program.

Subjects: 70 families with at least 2 cases of melanoma (1 of 8; 17 of 3 and 49 of 2) and 696 consecutive MM (292 males / 404 females) without previous familial history: 6 MPM (1), 4 MPM (3), 3 MPM (14), 2 MPM (64), only one primary MM (614).

Methods: Exon 1alfa, 1beta, 2, 3 and IVS2-105, -34G > T at the CDKN2A promoter region and EXON 2 from CDK4 were studied by PCR-SSCP analysis and sequencing; MC1R was studied by sequencing and c9orf14 variants by SSCP.

Results: CDKN2A mutations were detected in 24% of families being more frequent in families with multiple cases and 2,3% of sporadic melanomas, being more frequent in males (3.8% vs 1.2% p = 0.028), in patients with MPM (12.2% vs 1% p = 0.000), in patients with early age of onset (40 years-old or less) (5% vs 1.1% p = 0.001). Recurrent mutations were V59G, G101W, R87W, 358delG, D84Y, 102-106delG, R124C and -34G >. L65P was also present. The polymorphism A148T was present in 18% of families, 14.6% of MPM but only in 7.2% of non-MPM (p = 0.02).

A64. Genetic analyses of melanoma cell lines

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Objectives: Eleven human melanoma cell lines were genetically studied.

Methods: CDKN2A (p16 & p14ARF), CDK4 (exon 2), BRAF (exon 11 & 15) and NRAS (exon 1 & 2) were studied by SSCP and sequencing, BRAF mutator assay for V600E was also performed; MC1R genotype was studied by sequencing. By multiplex ligation dependent probe amplification assay (MLPA) we studied 30 human oncogenes (172 SALSA MLPA) and we finely mapped the region of 9p21 (SALSA MLPA P024B CDKN2A/2B region)

Results: We identified homozygous deletion (8 cell lines) and heterozygous deletions (2 cell lines) of CDKN2B region. The cell line without deletions has a homozygous mutation in CDKN2A. Four out of the cell lines presented one or more changes in MC1R and all of them had a V600E change in BRAF, this mutation is also present in another 2 cell lines. NRAS mutation is present in one cell line with an amplification of this locus. NRAS and BRAF mutations in these cell lines were mutually exclusive. CDK6 was amplified in 7 cell lines, CCND2 in 5 cell lines, the region 11q13-22 (including RELA, GSTP1, CCND1, EMS1, FGF3, BIRC3) in 2 cell lines and the region 20q11-13 in 5 cell lines. Finally, punctual amplifications of some oncogenes were identified (p.e. BIRC 5).We identified 66% (8/12) of samples with losses affecting 9p21 region and in all samples gains in at least one oncogene. In 9p21 the deletion was homozygous in 1 sample and heterozygous in 7 (in one case the deletion affected exclusively CDKN2B). Eight tumours presented one or more changes in *MC1R* (all were lower-risk red hair colour variants). One of them had the V600E change in *BRAF*. *STK15* amplification was present in 5 tumours; *CCND*1 amplification in 4 tumours and *NRAS*/*CDK4*/*EMS1*/*BIRC1* amplification were present in two tumours. One tumour had a point mutation in *ING-1* also present in lymphocytes from the same patient (germinal mutation).

A65. Gene expression profiling of cutaneous melanoma related to clinical outcome

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Although the prognosis for early local melanoma is favorable, distant metastasis represents 20–40% 5-year overall survival. Many clinical and histological features are related to cutaneous melanoma prognostic, but patients with similar features have different clinical outcome. We believe that molecular factors might be involved in this unexpected behavior. Aiming to identify possible genes related to cutaneous melanoma biology we established a data bank with clinical, histological and molecular informations from 63 patients treated in our service. Tissue samples from the tumors were extracted (23 primary and 40 metastatic lesions) to isolate RNA. Utilizing cDNA microarray technology, gene profile from each patient was achieved. This gene profile was used to compare groups of patients defined by the expected and non-expected prognosis and clinical evolution. Gene profile was also compared with patients prognostic factors as sex, primary tumor site, histological subtype, regression, ulceration, Breslow index and mitotic rate. AP3B2 and UGCGL2 were differentially expressed after comparing the groups. When analyzing the factor sex alone, JARID1D gene presented overexpressed in females.

A66. Genetic analyses of acral melanoma

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Twelve frozen samples from Acral Lentiginous Melanoma primary tumours were genetically studied.

Methods: *ING-1*, *BRAF* (exon 11 & 15) and *NRAS* (exon 1 & 2) were studied by SSCP and sequencing, BRAF mutator assay for V600E mutation was also performed; *MC1R* genotype was studied by sequencing. By multiplex ligation dependent probe amplification assay (MLPA) we studied gains or losses for 30 human oncogenes (172 SALSA MLPA) and the 9p21 region (SALSA MLPA P024B CDKN2A/2B region)

Results: We identified 66% (8/12) of samples with losses affecting 9p21 region and in all samples gains in at least one oncogene. In 9p21 the deletion was homozygous in 1 sample and heterozygous in 7 (in one case the deletion affected exclusively CDKN2B). Eight tumours presented one or more changes in *MC1R* (all were lower-risk red hair colour variants). One of them had the V600E change in *BRAF*. *STK15* amplification was present in 5 tumours; *CCND*1 amplification in 4 tumours and *NRAS*/*CDK4*/*EMS1*/*BIRC1* amplification were present in two tumours. One tumour had a point mutation in *ING-1* also present in lymphocytes from the same patient (germinal mutation).

Invited speakers

S1. CTLA-4 /PD1 pathways in regulating T cell responses and clinical trials data

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T cells are the central regulators of the adaptive immune response and their activation by antigen-presenting cells (APC) requires a first specific signal via the peptide presented by the MHC molecule, but also an additional signal involving engagement of costimulatory receptors on the surface of the T cell: CD28, with ligands expressed by APC, B7-1 and B7-2. To limit T cell activation, another molecule expressed on activated T cells: Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4 or CD152) can bind to B7-1 and B7-2 with a greater affinity than CD28 and down-regulate T-cell activation. Programmed-death-1 is another co-inhibitory molecule expressed on activated T cells, which binds to PD ligand (PD-L1) on APC, that has been shown to play a critical immunoregulatory role. Both CTLA-4 and PD-1 are also expressed on regulatory T cells and are physiological actors of peripheral tolerance.

Based on the hypothesis that these inhibitory pathways could also play an important role in cancer tolerance, molecules inhibiting CTLA-A or PD-1 were tested in several *in vivo* tumor models and showed encouraging antitumour activity. Drugs targeting CTLA-4 and PD-1 are now being developed and tested in cancer patients. PD-1 antibody is still in the early development phases, whereas two monoclonal anti-CTLA-A antibodies are already in phase III. One is developed by Pfizer (ticilimumab), and the other by Medarex/BMS (ipilimumab). Both gave promising efficacy signals in patients with melanoma in phase I clinical trials. Results of the phases II are pending and both drugs are now tested in phase III, in association with dacarbazine or with melanoma peptides. It is noteworthy that both antibodies are associated with adverse autoimmune side effects that might be correlated to the clinical antitumour response. Although there is a strong scientific rational to use these drugs in cancer patients, the exact mode of action of anti-CTLA-4 antibodies in humans and in particular the involvement of T reg remain uncertain and under active investigations.

S2. Subgroup analysis of efficacy and safety analysis of a randomized, double-blinded controlled phase 2 study of STA-4783 in combination with paclitaxel in patients with metastatic melanoma

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Background: STA-4783 (S), an inducer of heat shock protein 70 (hsp70) is a bis-thiobenzoylhydrazide compound. S leads to up-regulation of hsp70 in tumor cell lines. Xenograft models of solid tumors showed synergistic anti-tumor activity in combination with paclitaxel (P). The combination P + S, in Phase 1 and 2 studies, showed dose-related hsp70 induction (evidence of biological activity) and tolerability. **Methods**: Eligibility was based on a diagnosis of metastatic cutaneous melanoma, ECOG $< = 2$, and prior treatment with 1 or no chemotherapy regimens. A total of 81 patients (pts) were randomized 2:1 (P 80 mg/m^2 + S 213 mg/m^2:P 80 mg/m^2) 3 weeks out of 4 at 21 US clinical sites. The primary endpoint was progression free survival (PFS); secondary endpoints were response rate (RR), and adverse events (AEs). **Results**: Based on intent-to-treat analysis, the median PFS was 3.68 months (m) for P + S vs. 1.84 m in the P only arm ($p = .035$). RR was 15.1% in the P + S arm and 3.6% in the P arm. Subgroup analysis showed chemo-naïve pts ($n = 23$) with P + S showed a median PFS of 8.28 m vs. 2.40 in the P arm ($n = 9$). For pts with 1 prior chemotherapy, ($n = 29$), PFS on P + S was 3.12 m vs. 1.77 m on P ($n = 19$). Of 19 pts who crossed over at progression, data are available for 14. PFS ranged from 0.72 to 5.5 m. Three of the 14 evaluable pts treated with P alone had rapid progression (0.95, 1.6, and 1.7 m) then significant inversion of the time to progression with the addition of S to P (2.3, 5.5, and 4.2 m) suggesting study drug effect. Scans were done at identical intervals (8 weeks). The proportion of pts with AEs of grade 3 or higher was 54% ($n = 52$) in the P + S group and 57% in the P group ($n = 28$); pts on P received a median of 2 cycles, while pts in the P + S group received a median of 4. Adverse events leading to discontinuation were low in both groups: 10% for the P + S, and 14% for P.

Conclusions: The addition of S to P showed an increase in PFS vs. P alone particularly in chemo-naïve pts. A few pts failing single agent P appeared to benefit from P + S. Despite the additional treatment duration in the P + S group the drugs were well-tolerated, and showed mainly P related adverse events. A phase 3 study is planned to confirm a role for P + S in metastatic melanoma.

S3. Biomarkers

Ugurel S

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Biomarkers are important tools in clinical diagnosis and prognostic classification of various cutaneous malignancies. Besides of clinical and histopathological aspects (e.g. anatomic site and type of the primary tumour, tumour size and invasion depth, ulceration, vascular invasion), an increasing variety of molecular markers have been identified, providing the possibility of a more detailed diagnostic and prognostic subgrouping of tumour entities, up to even changing existing classification systems. Recently published gene expression as well as proteomic profiling data indicate new candidate molecules involved in skin cancer pathogenesis, which may after further validation represent new markers superior to existing ones. This ongoing process of biomarker identification and validation results in a rapidly changing molecular view and classification of skin cancers. It may be expected, that the rapidly increasing knowledge about molecular mechanisms leads to mainly biomarker-based rather than morphology-based classification systems, that may facilitate an individualized, molecular-driven cancer therapy.

S4. How the Sydney melanoma unit became a centre for multidisciplinary melanoma treatment and research

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The activities of the Sydney Melanoma Unit (SMU) commenced in the mid 1960's, when Dr Gerald Milton of the Department of Surgery at the University of Sydney recognised that there was a need for a special clinic for patients with melanoma [1]. Sydney is the largest city in Australia, a country with a very high incidence of melanoma, and the SMU soon became the major referral centre for melanoma patients from throughout New South Wales. Dr Milton also recognised that data collection was important to understand the disease better and improve treatment, and with the assistance of Dr Helen Shaw the SMU database was established. As patient referrals to the SMU increased rapidly in number, it became apparent that to provide complete melanoma care it would be necessary to offer chemotherapy, radiotherapy and palliative care, and steps were taken to provide these additional services. A domiciliary nursing service for patients with advanced disease was established, at a time when the value of such services had not been generally recognised. The research activities of the SMU were facilitated by the establishment of the Melanoma Foundation within the University of Sydney, providing funds for research infrastructure, as well as for public education. Approximately 1,300 new patients with melanoma are now referred to the SMU each year, and its database now contains information on over 25,000 patients.

The SMU provides integrated multidisciplinary care for patients with melanoma. This care is provided not only by surgical oncologists who undertake surgical treatment of patients with primary melanomas and recurrent disease but also by medical oncologists, radiation oncologists, nuclear medicine physicians, histopathologists, radiologists, palliative care specialists and oncology nurses, all with particular expertise and experience in the diagnosis and treatment of patients with melanoma. A weekly multidisciplinary clinical review meeting is attended by all SMU clinicians, nurses and clinical research staff.

This fosters not only a strong spirit of unity and cohesion but also a high level of adherence to agreed treatment guidelines and clinical trial protocols. At these meetings evidence-based treatment policies are developed, with input from all members of the multidisciplinary team.

The appropriate care of patients with melanoma is critically dependant on high calibre pathology, and the SMU was fortunate to have Dr Vincent McGovern as part of its multidisciplinary team in the early days, and subsequently Dr Stanley McCarthy and Dr Richard Scolyer, all internationally acknowledged experts in the pathology of melanocytic tumours. Over the past two decades, clinical trial activity at the SMU has expanded exponentially, with major contributions to important international multicentre studies. The willingness of SMU patients to enter these trials has been largely because they have been impressed by the skill, professionalism and dedication of the multidisciplinary team members who care for them. The SMU has identified key neurosurgeons and thoracic surgeons with whom it works closely, and medical oncologists, clinical immunologists, radiation oncologists, nuclear medicine physicians, radiologists and palliative care specialists are all part of the team. An offshoot of the SMU provides diagnostic services for pigmented skin lesions, and clinical and laboratory research is undertaken by SMU clinicians and researchers who are based at two other centres (Westmead Hospital in western Sydney and the Mater Hospital in the city of Newcastle).

The SMU provides a disease-specific specialist clinical service that has been highly successful. Key elements contributing to this success have been the sense of unity and purpose fostered by weekly multidisciplinary meetings, and the strong commitment of staff to clinical and basic research as a concomitant of high quality clinical care. Also of great importance has been the careful prospective collection of data, which has provided improved understanding of the natural history of the disease and has allowed the development and critical assessment of new treatment protocols.

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S5. The role of primary prevention in melanoma: 2007

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The incidence of melanoma (MM) continues to increase worldwide as does its associated mortality, especially in older men. Primary prevention and early detection remain the cornerstones of patient care. It is understood that ultraviolet (UV) radiation from the sun is the main environmental agent linked to the development of MM. We also know that most people are aware of the hazards of excessive sun exposure and yet they seldom take adequate precautions to reduce their UV exposure. These barriers to action may be augmented in part by the confusion promulgated by both the medical and lay press regarding a number of issues. These include the benefits and risks of several agents such as sunscreens, Vitamin D and sun exposure itself.

Sunscreens remain an important part of an overall sun protection program but they represent only one facet of the entire package. Even with the best sunscreens available today their efficacy is limited by a lack of awareness on the part of the consumer about the proper way to apply these UV filtering agents. In the past it was hypothesized that sunscreens contributed to the development of MM by allowing individuals to stay out in the sun longer without burning. A recent analysis of the literature suggests that sunscreens do not pose a risk for MM. However, the protective value of these products against MM formation remains to be determined. As both common and dysplastic nevi increase in number with age and proximity to the equator, it has been suggested that sunscreens may be able to reduce the incidence of these lesions (and thus serve as a surrogate for MM). To date, only one prospective study has been able to show a significant reduction in the incidence of melanocytic nevi in children who used a high SPF sunscreen over a three-year period. By contrast, clothing has been shown to effectively reduce the incidence of melanocytic nevi in children, and the incidence of MM is reduced in fair-skinned Caucasian adults who are heavily clothed for cultural reasons.

Recent data linking Vitamin D deficiency/insufficiency to a multitude of cancers, primarily in women, has lead to uncertainty as to whether or not sun exposure should be advocated for everyone. Suggestions that sun exposure may lead to a decreased mortality from MM confuses the situation even further.

Chemoprevention of MM has not been shown to be effective to this point in time and therefore, we should be advocating limited sun exposure, vitamin D supplementation, clothing, shade and sunscreens as the primary means by which we can reduce incident UV exposure in high risk populations. It remains to be seen whether other interventions such as increased physical activity will have any role to play in the reduction of skin cancers and MM in particular.

S6. Risk factors for melanoma development

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Defining the major risk factors of cutaneous melanoma (CM) is important for the identification of individuals at risk of developing the disease and for the optimization of primary and secondary prevention strategies. Risk factors of melanoma have been traditionally separated into genetic and environmental factors, although it is likely these factors are inter-related and that genetic features determining the cutaneous response to environmental stimuli, i.e., ultraviolet radiation, influence the susceptibility to melanoma (gene-environment interactions). In addition, recent evidence suggests that melanoma is a heterogeneous disease displaying different clinico-epidemiologic characteristics and developing through distinct molecular and pathogenetic pathways. The genetic nature of the disease is clearly demonstrated by the presence of a strong family history of melanoma in a small subset of patients (5–10%) and the genetic linkage with CDKN2A and CDK4 germline mutations in 25–40% of melanoma-prone families. Furthermore, patients with a previous history of melanoma carry a 3–6% risk of developing a subsequent melanoma. Numerous analytical studies have shown that the strongest risk factor of sporadic melanoma is the presence of multiple benign or atypical melanocytic nevi. Pooled relative risks demonstrate a 6-fold increased risk of melanoma in individuals with a large number of nevi (>100 nevi), while those having a moderate to large number of atypical (dysplastic) nevi (>5) harbor up to a 10-fold risk for developing melanoma, particularly in the context of a family history of the disease (atypical/ dysplastic nevus syndrome). In addition, large congenital nevi, sized $>$ 20 cm, have an estimated lifetime risk between 5 and 20% for CM. The main environmental risk factor of melanoma is excessive exposure of fair-skinned individuals to ultraviolet radiation (UVR), particularly in the form of natural sunlight. However, the exact wavelengths and patterns of ultraviolet exposure that contribute to melanoma remain speculative. Epidemiologic observations suggest that intermittent sun exposure increases risk presumably through stimulation of melanocytic activity and induction of genetic changes, whereas chronic or low-grade sun exposure induces protection against DNA damage through skin thickening and pigmentation. A specific set of pigmentary traits marked by sensitivity to UVR, such as fair skin, light-colored eyes and hair, skin phototype I and II, frequent sunburns and freckling have been associated with a modest increase (2-fold) of melanoma risk. Further risk factors include immunosuppresion (3-fold increased risk of melanoma in solid organ transplant recipients or patients with inherited immunodeficiencies) and rare types of genodermatoses characterized by defective DNA repair mechanisms, i.e., xeroderma pigmentosum. Other potential contributors to melanoma, but with a weaker strength of evidence, include exposure to artificial UVR sources (tanning beds), obesity, diet, higher socioeconomic status, male sex, industrial occupation, and hormonal factors (pregnancy, estrogen use).

S7. Low-penetrance melanoma susceptibilitygenes

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Susceptibility genes identified in families with melanoma (*CDKN2A* and *CDK4*) explain a very small proportion (1–3%) of melanoma in the population. Much ongoing research is focused on the identification of low-penetrance cutaneous melanoma susceptibility genes, conferring a lower melanoma risk with more frequent variations. The only gene that is clearly identified as low-penetrance melanoma susceptibility gene is *MC1R*, which encodes the melanocyte-stimulating hormone receptor. Polymorphic variants at this locus behave as the key determinants of the Red-Hair Color (RHC) phenotype characterized by red hair, light skin, poor tanning ability and heavy freckling. Several association studies have recently shown an influence of *MC1R* status on melanoma risk, even after adjustment for pigment variation. In Celtic populations, many of the *MC1R* variants associated with increased skin cancer risk are the RHC variants while in darkly-pigmented Caucasian populations non-RHC variants appear to influence melanoma risk as well. In the Italian population, carriers of one or more *MC1R* RHC variants are at increased melanoma risk and progression as compared with carriers of the wild-type sequence. Interestingly, separate analysis of individual *MC1R* variants shows that the increase in melanoma risk is restricted to the R151C and D294H *MC1R* alleles. Finally, the role of other candidate low-penetrance genes encoding proteins involved in pigmentation, cell growth and differentiation, DNA repair or detoxifying of metabolites has been addressed in several studies with conflicting results.

S8. The GenoMEL study of melanoma-prone families from three continents

Gruis, N.A. for GenoMEL (the melanoma genetics consortium)

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Over the past 20 years, the incidence of cutaneous melanoma has increased dramatically worldwide. A positive family history of the disease is among the most established risk factors for melanoma; it is estimated that 10% of melanoma cases result from an inherited predisposition. GenoMEL (formerly known as the Melanoma Genetics Consortium) comprising major familial melanoma research groups from North America, Europe, Asia, and Australia has created the largest familial melanoma sample yet. The mission of GenoMEL is to:

1. Identify melanoma susceptibility genes

Germline mutations in the CDKN2A gene, which encodes two proteins (p16 and p14ARF), are the most common cause of inherited susceptibility to melanoma, however they account for only 20%–25% of families with multiple cases of melanoma. Therefore, to localize additional loci involved in melanoma susceptibility, GenoMEL has performed a genome wide scan for linkage in pedigrees from several continents containing at least three melanoma cases, in which CDKN2A involvement has been excluded. Analysis provided significant evidence of a novel susceptibility gene for melanoma located within chromosome band 1p22 (Gillanders et al., 2003).

2. Assess the risk of melanoma and other cancers related to variations in melanoma genes

A GenoMEL study on the penetrance of mutations in the CDKN2A gene using data from groups from Europe, Australia and the United States revealed an overall CDKN2A mutation penetrance of 0.30 by age 50 years and 0.67 by age 80 years (Bishop et al., 2002). Penetrance was not modified by gender or by whether the CDKN2A mutation altered the p14ARF protein. However, penetrance varied with melanoma population incidence rates.

GenoMEL furthermore evaluated the relationship of mutations in CDKN2A and in the second melanoma gene, CDK4, with other cancers such as pancreatic cancer (PC), neural system tumours (NST), and uveal melanoma (UM) (Goldstein et al., 2006). Overall, 41% of families with at least three melanoma patients had mutations; most involved CDKN2A/p16. Mutations in CDK4 and CDKN2A/ARF occurred at similar frequencies (2–3%). There was a strong association between PC and CDKN2A mutations ($P < 0.0001$). In contrast, there was little evidence for an association between CDKN2A mutations and NST ($P = 0.52$) or UM ($P = 0.25$).

3. Evaluate gene-environment interactions

To further study determinants of melanoma, GenoMEL has developed a common questionnaire and database. The questionnaire is concerned with personal and family history of cancer, sun exposure and phenotypic data (nevus count, hair colour, freckling). These data in combination with mutation screening results will be used to perform genotype/phenotype and gene/environment interaction studies. The statistical power of these studies will be significantly increased as a result of the size of the data set and by including genetically diverse populations from very different latitudes.

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S9. Genetics and genomics in melanoma

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Advances made possible during the Human Genome Project have revolutionized our approach to tumor genetics and cancer genomics, including melanoma. Four cardinal translational goals for all melanoma researchers include

1. **The identification of high risk individuals**. Since the first description of germline CDKN2A mutations in a subset of melanoma patients over a decade ago, much progress has been made in our understanding of high risk and moderate risk loci. Whole genome association studies are underway in all diseases including melanoma. Over the next five years, we can anticipate a systematic disclosure of multiple melanoma risk alleles through these studies although the clinical implications of this information remain to be established.
2. **The identification of high risk lesions**. Technologies in molecular imaging and optics will allow us to visualize the invisible and refine phenotypes beyond the traditional cutaneous language.
3. **The identification of higher order molecular structure in melanoma**. Our view of melanoma remains largely histological. Prognosis and classification are still based on morphometric criteria although an ever-expanding collection of "mel-anomalies" (eg. atypical Spitz tumors, pigmented melanocytomas) challenges our fundamental assumptions about metastasis and malignancy. Genome-wide approaches at classifying melanoma may soon uncover behavioral correlates that are currently not recognizable.

4. **The identification of highly effective therapies**. With the recognition that the MAP kinase signaling pathway is activated in a majority of melanomas by oncogenic activation of either NRAS or BRAF, there is an intense effort by both the scientific and pharmacological communities to rapidly deploy clinically-viable products.

S10. Dermoscopy in the diagnosis of initial melanomas

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In the last two decades a rising incidence of melanoma has been observed. Owing to the lack of adequate therapies for metastatic melanoma, the best treatment currently is still early diagnosis and prompt surgical excision of the primary tumor. The current practice in the diagnosis of melanoma is based on the ABCD rule, which uses four simple, clinical, morphologic features of melanoma (*A*symmetry, *B*order irregularity, *C*olor variegation, and *D*iameter more than 5 mm). There are, however, two major problems with the current practice of clinical diagnosis of melanoma. First, clinical diagnosis based on the ABCD rule reaches only 65% to 80% sensitivity because this method does not recognize that small melanomas (less than 5 mm) may occur. In addition, very early melanomas may have a regular shape and homogeneous color; such lesions would falsely be assessed as benign. Second, numerous unnecessary excisions may be performed, since a number of benign melanocytic nevi may mimic melanoma from a clinical point of view. Dermoscopy can help overcome these problems and is a useful addition to clinical diagnosis.

The introduction of dermoscopy into the clinical practice of dermatology has disclosed a new and fascinating morphologic dimension of pigmented skin lesions (PSL). Dermoscopy is a non-invasive, simple, and inexpensive diagnostic technique that permits the visualization of morphologic features that are not visible to the naked eye, thus forming a link between macroscopic clinical dermatology and microscopic dermatopathology. This sub-macroscopic observation of PSL enriches the available clinical diagnostic tools by providing new morphologic criteria for the differentiation of melanoma from other melanocytic and non-melanocytic PSL.

Key points for differentiation will be discussed and several dermoscopic hints not to miss melanoma will be especially emphasized.

S11. Challenges for dermoscopy and non-invasive imaging in melanoma diagnosis

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A complete revolution in the imaging technology for melanoma diagnosis is at our doorstep. Techniques that allow a detailed, immediate, noninvasive evaluation of the skin at the bedside are now available. Among all the techniques for imaging in dermatology we will review total body-photography, dermoscopy, digital follow-up and confocal reflectance microcopy in vivo based on the experience of the Melanoma Unit of the Hospital Clinic of Barcelona.

Total body photography, already recognized for its usefulness in the early detection of melanoma, has been subject to major technological improvement. Systems for the computerised assisted detection of new or changing may improve skin cancer surveillance in patients with risk for melanoma in atypical mole syndrome. In patients with multiple atypical nevi the confirmation of stable lesions during follow-up or the observation of changes is a useful tool in the clinical setting.

Dermoscopy, dermatoscopy or epiluminiscence improved the accuracy in the early detection of melanoma in multiple studies and in two published metanalyses. This technique is now used by dermatologists in all the continents for the examination of the pigmented and non-pigmented lesions. The incorporation of higher magnification microscopes to the practice of dermoscopy allows for studying skin lesions in-vivo in greater detail. Moreover ex vivo dermoscopy may serve to guide tissue sectioning in gross pathological evaluation and also for the fresh tissue sampling for molecular studies.

Reflectance-mode confocal microscopy (RCM) produces horizontal images of the skin at a maximum depth of 300 microns at a cellular level resolution. This technique allows the precise examination of the skin structures at different depth level starting from the surface to the papillary dermis. RCM has been employed for the study of normal skin and different diseases, resulting also promising in the differential diagnosis of MMs with other pigmented or non-pigmented tumors of the skin. Confocal microscopy is the natural link between dermoscopy and histopathology. In clinical practise RCM allows the recognition of subtle melanomas, helps to establish surgical margins even in amelanotic tumors and it can support the detection of recurrences to previous treatments.

S12. The use of tissue microarray as a tool to evaluate the importance of cell cycle proteins, integrins and metallo-proteinases in melanoma behaviour

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In the last few years, molecular biology allowed to describe new genes and proteins, and have challenged the capacity of scientists to determine their value as markers and prognostic factors. Thus, high throughput techniques were developed to study in a single experiment, large number of specimens. Tissue microarray (TMA) is one such technique. Representative samples of tissue obtained from paraffin blocks are organized in an array, and have been built to validate protein markers, to establish prognostic factors, to use as multiple organ controls, and also to evaluate gene expression with FISH and CISH, among other utilities.

TMA has been used to study preferentially solid tumours, for sampling are frequently available due to the size of lesions. Very small lesions add some difficulty to obtain representative tissue to be used in a TMA. The sections may not have all depth of the tumour, or it may well be that some irregularity in distribution and format of that particular area would lead to incomplete sampling. This is why TMA for melanoma has some drawbacks, though very important papers have been published in this field.

Investigative design:

In order to investigate the expression of cell cycle, cell death, and other proteins, two series of melanoma have been evaluated either with TMA and conventional sections.

The first approach was to select only in situ and invasive superficial spreading melanoma, combining in the same TMA, different depths of primary melanomas and as far as possible, their respective metastases (subcutaneous, lymph node and lung). By the end of the study, all patients had at least 4 years follow-up, a feature that allowed establishing the protein expression and its relevance in the behaviour of melanoma.

The second approach aimed to study a smaller group of patients that had been evaluated for sentinel lymph node. Patients with positive and negative micrometastatic disease were selected and their primary lesions were included in the TMA. In this group, other than cell cycle protein expression, metalloproteinases and alpha-v-beta3 integrin were also evaluated. Again, minimal follow up was 4 years by the end of the study.

First approach: Superficial spreading melanoma

We analyzed the expression of cell cycle and apoptosis proteins in samples of cutaneous in situ and invasive superficial spreading melanoma aiming to evaluate and establish the correlation with the biological behaviour and site of metastases. Patients and methods. A retrospective study of 136 patients with cutaneous superficial spreading melanoma, diagnosed and treated at the Hospital A C Camargo. Paraffin blocks of primary and metastatic tumours were obtained from the files of the Anatomic Pathology Department. A total of 64 samples of in situ and less than or equal to 1,0 mm melanomas were analysed in conventional sections. Melanomas over 1,0 mm were evaluated in the TMA, including 72 samples of primary and 29 from metastatic tumours. Two samples of each paraffin blocks were included. The sections were stained with antibodies against p16,

cyclin D1, Cdk4, pRb, p53 and p21 with streptavidine-biotin-peroxidase technique for immunohistochemistry. Results. Melanomas above 1,0 mm lost p16 expression whereas in situ e and thin melanomas had low rate of expression. On the other hand the latter showed higher expression of Cyclin D1 and cytoplasmatic Cdk4. Thick melanomas had increased expression of nuclear Cdk4, p53 e p21. No relationship to thickness has been found for pRb. Ulceration and regression had no association with any of the studied proteins. Melanomas with MR ≥ 1 had lower cyclin D1 expression, whereas p53 had higher expression in melanomas wilh MR > 6. Primary tumours, when compared to metastases had higher cytoplasmatic Cdk4 expression. Cutaneous compared to non cutaneous metastases had higher p21 expression. None of the studied proteins had influence in overalll or disease free survival. Conclusions. Our results allow concluding that loss of p16 expression was a constant feature in these series. Cyclin D1 could be related to initial phases of the malignant transformation. Thin melanomas would have higher expression of cytoplasmatic Cdk4 whereas thick melanomas acquiring other mutations would gain nuclear expression of this protein. Little influence on biological behaviour and pathogenesis of cutaneous melanoma appears to be related to pRb, whereas over expression p53 may be a late phenomenon. An increase in p21 could represent a cell cycle feedback control for cells that lack or lose p16 and/or increase nuclear Cdk4 expression.

In the same setting of TMA, in situ melanoma had an intense $\alpha v\beta 3$ integrin expression. This intriguing finding suggests that, before invading the dermis, melanoma cells may have to express some factors that would allow interaction with extracellular matrix, among which $\alpha v\beta 3$ integrin could be one such factor. In fact, a relationship between $\alpha v\beta 3$ and ras/raf/MEK/ERK pathway has been reported, indicating that prior to the engagement with extracellular matrix a binding to ERK1 is a necessary step to establish a link to vitronectin, a basement membrane constituent and ligand to $\alpha v\beta 3$. Moreover, metalloproteinases such as MMP-2 and MMP-9 and CEACAM1 are involved in cell invasion and migration that appears to be modulated by $\alpha v\beta 3$ expression.

These results led us to further investigate another setting of patients, the ones that had sentinel lymph nodes investigated for micrometastases.

Second approach: Sentinel lymph node and primary melanoma

It has been established that SLN is one of the most accurate methods to establish prognosis for melanoma and other diseases, such as breast cancer, and is a worse outcome predictor. However, very few studies have compared the expression of cell cycle proteins to establish a link with micrometastatic melanoma. To address this issue, our group evaluated the expression of cell cycle proteins (Cyclin D1, CDK4, p16ink4, p21WAF1), cell adhesion protein ($\alpha v\beta 3$ integrin) and proteolythic enzimes through activation of metalloproteinase-2 (MMP-02) and metalloproteinase-9 (MMP-09) in specimens of primary cutaneous lesions of melanoma that did and did not metastasize to SLN, using immunohistochemistry. Patients and methods: this is a retrospective study of 84 patients diagnosed as primary cutaneous melanoma in the Department of Anatomic Pathology of the Hospital A C Camargo, that had been evaluated for SLN between 1998 and 2002. Immunohistochemistry was performed in both, conventional glass-slide sections for less than 1 mm melanomas (17 specimens) and Tissue micro array (TMA) glass slides. The TMA paraffin block was built with 134 samples of melanoma with 1 mm cores (67 patient melanomas in duplicate). Twelve melanocytic nevi were included as a control.

Results: Patients had mean age of 53 years-old with slight women predominance (53%). SLN was positive in 20 (24%) patients, and negative in 64 (76%) patients. An association with a positive SLN was obtained when evaluating Breslow's tumour thickness (p = 0,011), Clark's level (p = 0,010) and perineural invasion (p = 0,011). Only the absence of cyclin D1 expression had statistical significance to correlate with SLN micrometastases (p = 0,001). All other molecular variables had no relationship with SLN micrometastases. When multiple logistic regression was applied, Breslow's thickness (OR = 0,011; IC95%:1,7–63,7), perineural invasion (OR = 0,013; IC95%:1,9–318,9) and negativity for cyclin D1 (OR = 0,010; IC95%: 1,5–22,8) had significant association with metastatic disease in the SLN. Although we have found a correlation between $\alpha v\beta 3$ integrin expression and MMP-2 has been found, these proteins did not have correlation with the development of nodal metastases. All intradermal melanocytic nevi expressed p16. No expression of p21 was found.

Conclusions: Breslow's thickness of melanoma is still the major prognostic factor to predict metastatic behaviour in SLN. It is likely that the absence of cyclin D1 is a prognostic factor for the development of SLN metastases, for both, univariate and multivariate analysis pointed out a relationship between cyclin D1 absence and SLN metastases. CDK4, p16 and p21WAF1 does not seem to have an impact as a predictor for nodal metastasis. $\alpha v\beta 3$ integrin expression and MMP-2 could not predict a metastatic behaviour.

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S13. EORTC 18991: long term adjuvant pegylated interferon-α2b (PEG-IFN) vs observation in resected stage III melanoma: final results of a randomized phase 3 trial

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Background: EORTC 18991 is the largest adjuvant trial ever conducted in stage III melanoma. It assessed the efficacy and toxicity of long term PEG-IFN vs Observation (Obs.).

Methods and Patients: High dose PEG-IFN (Induction at 6 μg/Kg/wk, sc, 8 weeks; followed by Maintenance at 3 μg/Kg/wk, sc) for 5-years total treatment duration was compared to Obs. in 1256 patients (pts) with stage III melanoma (anyTN1-2M0 without in-transit metastases). Randomization was stratified for nodal involvement N1 (microscopic) vs N2 (palpable nodes), # of nodes, Breslow, ulceration of primary, sex and center. Distant Metastasis-Free Survival (DMFS) was the primary endpoint. Relapse-Free Survival (RFS) was the pre-specified regulatory primary endpoint. Overall survival (OS) was the secondary endpoint. Intent-to-treat analysis was performed.

Results: Median follow-up was 3.8 yrs:

	RFS		DMFS		OS	
	Obs.	PEG-IFN	Obs.	PEG-IFN	Obs.	PEG-IFN
Nb. events	368	328	325	304	263	262
4-year rates	39%	46%	45%	48%	56%	57%
Median (yrs)	2.1	2.9	3.0	3.8	NR	NR
HR (95%CI)	0.82 (0.71-0.96)		0.88 (0.75-1.03)		0.98 (0.82-1.16)	
p-value	0.01		0.11		0.78	

HR Hazard ratio; *NR* not reached

In N1-pts (n = 543) the benefit of PEG-IFN seemed more pronounced than in N2-pts (n = 713): RFS (HR 0.73 p = 0.02 and HR 0.86 p = 0.12 for N1 and N2, respectively), DMFS (HR 0.75 p = 0.03 and HR 0.94 p = 0.53) and OS (HR 0.88 p = 0.43 and HR 1.01 p = 0.91).

PEG-IFN treatment relative dose intensity (actual/planned dose while treated) reached median 88% (induction) and 83% (maintenance). 251 pts (40 %) stopped PEG-IFN because of toxicity (31%) or refusal for other reasons 9%. Grade 4 toxicities occurred in 9% (PEG-IFN) and in 7% (Obs.) indicating that these were mostly not treatment realated events. Grade 3 toxicities were reported in 38% (PEG-IFN), vs 9% (Obs.), including most frequently fatigue (15%), hepatotoxicity (10%) and depression (6%) with ECOG 0-1 Performance Status maintained in 82% (induction) and 86% (maintenance) of pts.

Conclusions: Long term high dose PEG-IFN therapy in stage III melanoma had a significant and sustained impact on RFS, but not on DMFS and OS. However in Pts with only microscopic nodal involvement (Sentinel Node positive) the impact of PEG-IFN was significant on bothe RFS (HR 0.73, p < 0.02) and DMFS (HR 0.75; p < 0.03). Similar better effects of adjuvant IFN therapy in pts with lower disease burden were observer in the EORTC 18952 trial and are consistent in 2 consecutive EORTC trials (18952 and 18991) involving 2644 pts, indicating sensitivity to IFN therapy for the N1 population.

S14. Shall we perform sentinel node biopsy if benefit for overall survival is lacking?

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Sentinel lymph node biopsy (SLNB) or selective sentinel lymphadenectomy is a reliable technique to assess the presence of metastasis in the nodal basin of the primary melanoma. The SLN status provides accurate prognostic information for patients with primary melanoma based on multiple large series (Leong et al. WJS 2005;29:683) with the SLN-negative patients having a much more favorable outcome. Although the randomized multicenter selective lymphadenectomy trial (MSLTI) did not show the therapeutic benefit of SLNB (Morton et al. NEJM 2006;355:1307), it identifies an intermediate-thickness subgroup (1.2 to 3.5 mm) with nodal metastasis whose survival was prolonged by immediate lymphadenectomy. Further, SLNB serves as a crucial stratification criterion for clinical trials as demonstrated in the EORTC study reported in the recent ASCO meeting by Eggermont et al (2007, Proceedings 25 (18S):8504) that patients with only SLN-positive seemed to have better outcomes, following long term PEG-IFN treatment, in terms of both relapse-free and distant metastasis-free survival. Therefore, SLNB is an appropriate staging procedure for patients with primary melanoma. Perhaps, in the future, the criterion for SLNB may be better defined with inclusion of more accurate prognosticators of the primary melanoma for SLN metastasis.

S15. The role of surgery in stage IV melanoma

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Recent prospective trials have shown that patients undergoing complete resection of stage IV melanoma have longer disease-free and overall survival than would have been expected with currently available systemic therapy. But since most patients with metastatic melanoma have disease that is too extensive to resect or is unresectable by virtue of its location, it is clear that selection plays a major role in achieving good results with surgical metastasectomy. Patients with isolated, potentially resectable distant metastases most often harbor microscopic metastases that preclude long-term disease control by the initial surgery. Advances in imaging technologies as well as improvements in surgical techniques and perioperative care raise the prospect that patients with truly isolated metastatic disease can be identified and safely subjected to surgery, sometimes on a repeated basis to achieve long term survival even if the initial disease-free interval is relatively short. A surgical approach requires an understanding of the disease process, adequate imaging, and careful patient selection. Retrospective reports have noted that a long disease-free interval from primary diagnosis to the diagnosis of metastases, fewer metastatic lesions, and limited numbers of organ systems involved by disease all identify patients that are more likely to have prolonged survival after surgical intervention, but also after systemic therapies as well. While it is likely that someday high-throughput techniques for gene expression analysis will predictably identify patients who will benefit from surgical intervention as well as those that will not, for now clinical predictors must be used in the selection of patients with metastatic melanoma for surgery.

For patients with stage IV melanoma being considered for surgical treatment, the rate of identification of clinically occult metastases is high enough to justify extensive imaging studies prior to attempting surgery. Prospective data supports brain MR, and PET/CT scanning as worthwhile in the preoperative evaluation of stage IIIB/C and IV melanoma patients. Whole body PET/CT fusion scanning has been shown to be superior to whole body MR imaging, but still is associated with a substantial number of false positives. Glucose is readily concentrated in melanoma; however, the PET scan does have limitations. As with all imaging studies, the sensitivity of PET scans diminishes for detecting tumors less than 1 cm in size. Because PET is a purely functional study, anatomic localization can be problematic. To improve the anatomic localization, PET is combined with a CT scan, but hypermetabolic areas on PET with no corresponding CT abnormality are not unusual and can be difficult if not impossible to categorize as true or false positive findings. The presence of brain metastases, while not an absolute contraindication to surgery, generally leads to a decision not to operate on a patient with non-CNS metastases until and unless the CNS disease is controlled. Gadolinium-enhanced MR is the most sensitive test for detecting brain metastases from melanoma currently available, and should be routinely employed. Head CT is an alternative in patients with a contraindication to MR. Standard 18F-fluorodeoxyglucose PET is a relatively insensitive test for identifying brain metastases due to the brain's high glucose utilization.

When surgery is undertaken, complete resection of all disease should be the goal; there is little or no role for "debulking" operations that leave gross disease behind. Palliation is also rarely achieved long-term unless all gross disease is removed. A possible exception is actively bleeding intestinal metastases, where removal of all or most of the intestinal tumors can lead to reasonable palliation even if non-intestinal disease remains. Two recent prospective studies (SWOG-9430 and the Canvaxin adjuvant vaccine trial MMAIT-IV) show that a high percentage of patients who are candidates for surgery based on preoperative imaging go on to complete resection, and that a small but significant percentage of these patients survive disease-free for five years. These studies have also shown that there is significant selection bias after surgery for patients who are entered onto adjuvant trials, resulting in inherently better outcomes for trial participants than for unselected controls or for patients not undergoing protocol therapy. Interestingly, in both prospective studies, the median time to disease progression was relatively short (6 months in SWOG-9430, 7 months in MMAIT-IV 'placebo' arm), even though median overall survival times were much longer (21 months in SWOG-9430, 39 months in MMAIT-IV 'placebo' arm). While this is due in part to re-resection at the time of first post-surgical relapse, it is likely that a more indolent natural history of resectable stage IV melanoma influences the

outcome results. It may even be the case that patients who are selected for surgery have disease that is inherently more indolent, or perhaps inherently more sensitive to systemic therapies such as immunotherapy. If so, more aggressive use of adjuvant therapy might prolong the relapse-free and overall survival of patients after resection of stage IV melanoma, although the failure of MMAIT-IV to show a significant benefit for adjuvant use of an allogeneic polyvalent melanoma vaccine was disappointing.

Another setting in which surgical metastasectomy should be considered is in metastatic melanoma patients who have responded to systemic therapy with a complete response of most lesions but have one or two potentially resectable tumors remaining. Many single-institution reports note prolonged disease-free and overall survival when such patients are converted to a ''surgical complete response.'' This experience suggests that there may be also a role for neoadjuvant therapy in patients who are considered poor candidates for resection initially. Biologic correlative studies are urgently needed to determine if there is a ''biosignature'' that corresponds to favorable outcome after surgery and/or systemic therapies, and if there is such a signature whether other melanoma patients who are not currently considered good candidates for surgery might in fact benefit from aggressive resection of their disease. Surgery is an important option for carefully screened and selected patients with limited metastatic disease, and re-resection should be employed when feasible. Patients with resected stage IV melanoma should be entered into clinical trials of adjuvant therapy in hopes of increasing the percentage of patients who remain disease-free after surgery.

S16. Targeting signal transduction pathways in melanoma

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The elucidation of aberrant signaling pathways in melanoma has provided a rationale for the investigation of signal transduction inhibitors in advanced melanoma. Several secreted growth factors have been implicated in the pathogenesis of melanoma, including FGF, HGF, VEGF, and PDGF. Intracellular signaling abnormalities have been well-described in the MAP kinase and PI3 kinase pathways. The presence of activating mutations in NRAS and BRAF provide obvious targets for inhibition. The overactivity of the PI3 kinase pathway, as a result of PTEN loss, provides less defined targets. Given the multiplicity of mutations and subsequent signaling abnormalities in melanoma, clinical efficacy in melanoma will likely require the simultaneous inhibition of several critical targets with particular attention paid to subgroups of patients with coordinated sets of mutations. While the number of potential therapeutic targets has multiplied in recent years, the strategy for maximizing efficacy with signal transduction inhibitors has yet to be determined. The prevalence of BRAF mutations has drawn attention to the clinical evaluation of RAF and MEK inhibitors, alone and in combination with chemotherapy. Sorafenib was the only available RAF kinase inhibitor in 2002 when BRAF mutations were identified in melanoma. Evidence of activity in combination chemotherapy led to two randomized trials, one of which suggested activity with sorafenib in chemotherapy naïve patients, the other showing no activity with sorafenib among chemotherapy refractory patients. Trials combining sorafenib, temsirolimus, bevacizumab and tipifarnib are underway, however this list of agents is not entirely matched to the list of rational targets defined by the genetic evidence. The largest randomized trial with sorafenib, E2603 continues to accrue chemotherapy naïve patients. Novel BRAF inhibitors are currently in phase I/II testing and represents the leading edge of targeted therapies for melanoma.

S17. Malignant melanoma – molecular targets other than signal transduction proteins

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Chemotherapeutic alkylating agents such as temozolomide, dacarbazine (/DTIC) and carmustine (BCNU) generate a multitude of different types of lesions in DNA. Cytotoxicity can result from alkylation of the O^6 position of guanine residues. The DNA repair protein, MGMT is known to be important in tumour resistance to these drugs. O^6-(4-bromothenyl)guanine (lomeguatrib) and O^6-benzylguanine are MGMT pseudosubstrates that alkylate the active site cysteine residue of MGMT causing irreversible inactivation. Pre-treatment with either of these agents can thus reduce resistance to alkylating agents. Both lomeguatrib and O^6-benzylguanine are in clinical trials at the present time.

There is evidence that one of the other toxic DNA lesions, 3-alkyladenine (3AA), and its repair processes alkylpurine-DNA glycosylase (APG), might play a much more significant role in alkylating agent chemotherapy than was previously thought. 3AA is considered to kill cells by acting as a block to DNA replication and the formation of DNA single and then double strand breaks. APG combats this by removal of the modified base: the resulting apurinic site is processed by abasic site endonuclease (APE) and this generates strand breaks that can result in the activation of poly-ADP-ribose polymerase (PARP) which engages the other components of the long patch base excision repair (BER) pathway. The extent to which APG is an important factor in chemotherapy involving alkylating agents has yet to be established.

Polyadenosine 5'-diphosphoribose [poly-(ADP-ribose)] or PAR polymerisation is a unique post-translational modification of histones and other nuclear proteins that contributes to the survival of injured proliferating cells following DNA damage. The enzymes that catalyse this process, poly (ADP-ribose) polymerases (PARP), are critical regulatory components in DNA damage repair. They comprise an expanding family of 18 proteins, encoded by different genes and displaying a conserved catalytic domain. The range of biological roles of PARP is wide. PARP is involved in base excision repair of DNA damage: inhibition of PARP may also sensitise tumours to the effects of temozolomide and other alkylating agents. Preliminary results from a phase II study of AG014699 12 mg/m^2 and full dose temozolomide in patients with advanced MM showed significant activity (response and stabilisation) but also significant myelosuppression.

Since PARP-1 activation leads to DNA repair through the base excision repair (BER) pathway, cells deficient in PARP-1 have delayed DNA repair. PARP-2 also responds to DNA damage and its inactivation promotes apoptosis. Loss of PARP activity in cells or in knockout mice leads to both radio- and chemo-sensitisation. Moreover, increased PARP activity has been found in many tumour types. PARP inhibition can inhibit malignant cell growth and viability and can enhance the antitumour activity of radiation and DNA damaging cytotoxics. BRCA -/- tumours (both BRCA1 and BRCA2) are sensitive to PARP inhibition.

These potential therapeutic targets will be discussed in detail.

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S18. Sorafenib in metastatic melanoma

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Metastatic melanoma is associated with a median survival of 6–7 months, and a low (6%) 5-year survival rate. Complete responses are extremely rare and current treatments offer a median progression-free survival (PFS) of 1.8–4 months. Oncogenic b-raf mutations (mostly V600E) occur in 66% of melanomas. Sorafenib is a multi-kinase inhibitor that targets V600E b-raf, Raf-1, wild-type B-Raf, and VEGFR-2/-3, PDGFR-ϐ, c-Kit, Flt-3 and RET kinases in vitro. Both sorafenib and a b-raf V600E siRNA inhibit melanoma growth in melanoma xenograft models. In clinical trials, sorafenib has shown evidence of activity against several tumor types, including melanoma, and has been well tolerated both as a single or combination agent.

Several studies have been conducted with combinations of sorafenib and chemotherapy in patients with metastatic melanoma. Flaherty and colleagues conducted a Phase I/II trial of oral sorafenib

administered in combination with carboplatin/paclitaxel in 105 patients with advanced melanoma. Sorafenib was administered from Day 2 to 19 of a 21-day cycle at three dose levels: 100 mg, 200 mg and 400 mg bid. Carboplatin (AUC 6) and paclitaxel (225 mg/m^2) were co-administered on Day 1. The overall response rate was 26% with stable disease in 56% for an overall disease control rate of 85%. The median PFS was 8.8 months.

Agarwala and colleagues reported recently on the results of the PRISM trial, a randomized phase III study of the above regimen of carboplatin, paclitaxel and either sorafenib or placebo in 270 patients who had progressed on a first line dacarbazine or temozolomide containing regimen. The median PFS on the sorafenib arm was 17.4 weeks versus 17.9 weeks on the placebo arm and the response rate was 12% vs. 11% (p = ns). A similar trial in first-line metastatic melanoma patients is being coordinated by ECOG (E 2603).

McDermott and colleagues reported on the results of a randomized phase II trial of dacarbazine 1 gm/m2 every 21 days with sorafenib or placebo in 101 patients with metastatic melanoma who had received on prior therapy (first-line). PFS on the sorafenib containing arm was 21.1 weeks vs. 11.7 weeks for placebo (p = 0.06) and response rates improved to 24% vs. 12% (p = 0.19), showing a strong trend for benefit for sorafenib with DTIC as compared to sorafenib and placebo.

Conclusions: Sorafenib is an interesting and active inhibitor of b-raf with a strong rationale for testing in melanoma. Results of a randomized phase III trial in second-line therapy for metastatic melanoma combining sorafenib with carboplatin and paclitaxel did not show a benefit in PFS or response rates for sorafenib as compared to placebo. However, a trial in first line therapy of melanoma using dacarbazine showed a strong trend to improvement in PFS and RR for the sorafenib containing arm as compared with placebo. Ongoing studies such as E 2603 will establish the true role of sorafenib in combination with chemotherapy in this disease.

S19. How to individualize systemic therapies in melanoma

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Most medicines including the therapy of metastatic melanoma are currently prescribed empirically. However, advances in understanding the mechanisms of the disease, as well as drug response, are increasingly creating opportunities to match patients with therapies that are more likely to be effective and safe.

At the extreme of patient matching are 'individualized' medicines, which vary inherently for each patient. Cancer vaccines which are produced using tumour cells obtained from the individual patient during surgery, thereby representing the unique antigen 'signature' of that patient's cancer, exemplify such an individualized medicine.

An intermediate step between individualized and empirical approaches are therapies which are matched with specific patient population characteristics using clinical biomarkers. To this end, the pharmacogenetics of either individual tumours or subgroup of tumours can be used to aid the choice of anti-tumour drugs that are active against tumors characterized by aberrations of particular growth factor receptors. For example, the high frequency of activating mutations in KIT in melanomas on mucosal membranes or acral skin suggests a possible benefit for these patient groups if treated with the respective kinase inhibitor such as imatinib. Similarly, the pharmacogenetics of the individual patients with respect to polymorphisms in immune response or DNA repair genes may help to define patients who are more likely to benefit from an immune or genotoxic therapy, respectively.

The potential to use biomarkers for identifying patients that are more likely to benefit or experience an adverse reaction in response to a given therapy, and thereby better match patients with therapies, is anticipated to have a major effect on both clinical practice and the development of new drugs and diagnostics. However, the introduction of pharmacogenetic tests into routine healthcare requires both a demonstration of cost-effectiveness and the availability of appropriate accessible testing systems.

S20. Immunotherapy of melanoma with cell line derived antigens

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There is considerable evidence that immune responses play an important role in the evolution of melanoma. This has led to many trials using vaccines in an attempt to utilise immune responses against melanoma in treatment of the disease. The antigen source has varied from whole melanoma cells, to lysates of melanoma, to proteins, peptides and DNA coding for melanoma antigens. Various platforms of administration have been used. Nevertheless as will be shown, results of these vaccine trials have been disappointing with relatively few responses and minimal effects on measures of survival. Our studies in the SMU and NMU are representative of many of these studies. In some instances the poor results have simply reflected over optimistic results from phase 2 trials that have been compared to historical controls. In others the patient numbers were too small to detect small but meaningful differences in survival.

From the perspective of vaccine development remaining options consist of attempts to target defined antigen vaccines to patients with tumours known to contain the antigens. This also allows dose finding studies to be carried out. Examples include the NY-ESO-1 /ISCOMS trial being conducted by the Ludwig institute and the proposed phase 3 MAGE-3/AS15 trial by GSK biologicals. Secondly, studies are yet to be carried with vaccines against proteins in melanoma that are essential to the neoplastic process. This would prevent emergence of antigen loss variants as loss of the protein would mean death of the cell. Thirdly, combining vaccines with agents that inhibit regulatory T cells is yet to be evaluated in well controlled studies. Whether antibodies against CTLA4 will have such a role remains controversial.

It is possible however that new hypotheses need to be tested before immunotherapy can be considered an important treatment modality. Paramount amongst these is a stronger focus on the melanoma cell. Eg it is becoming clear that melanoma can condition its microenvironment and inhibit or direct immune responses into TH2 or TH17 inflammatory non cytotoxic responses. Secondly melanoma can be selected (by the immune system) to have strong anti apoptotic mechanisms that limit the ability of the immune system (or chemotherapy) to kill melanoma cells. Induction of Endoplasmic Reticulum (ER) Stress responses in melanoma are an additional cause of resistance of melanoma to cell death. These ideas imply that the way forward is combination of immunotherapy with agents that condition the melanoma cell. Fortunately many such agents are already in clinical use and it will be possible over the next few years to test these hypotheses.

S21. CTLA4 antibodies

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Cancer cells exploit immune regulatory checkpoints to grow largely ignored by the immune system. The cytotoxic T lymphocyte antigen 4 (CTLA4) is a main negative regulator of the immune system, which inhibits the costimulatory signaling for T cells provided by CD80 (B7.1) and CD86 (B7.2). Blocking antibodies against CTLA4 would release this negative signaling, and were shown to be able to induce regression of immunogenic tumors in mice [1].

Two fully human CTLA4 blocking monoclonal antibodies (generated in mice that had human immunoglobulin genes knocked-in), are in late stages of clinical development. Ipilimumab (formerly MDX010) is an IgG1 developed by Medarex Inc. that entered clinical testing in 2000, and is currently in joint clinical development with Bristol-Myers-Squib. CP-675,206 is an IgG2 from Pfizer Inc., which was transiently called ticilimumab, a name that had to be discontinued due to similarity with another monoclonal antibody. It first entered clinical testing in 2002.

Early clinical data demonstrated that both antibodies lead to objective durable tumor regressions in a subset of patients with metastatic melanoma. Across several clinical trials, including dose escalation, single dose, multi-dose, and in combination with a variety of

other immune stimulants like peptide vaccines or interleukin-2, objective tumor responses in the range of 10 to 20% were obtained in patients with in-transit and metastatic melanoma [2–11]. The most sticking characteristic of these responses is that most are durable, ongoing for several years.

These responses are at the cost of inflammatory or immune-mediated toxicities, which would be expected as effects related to the release of a major negative signaling pathway of the immune system. These include colitis and skin rash as the most common toxicities, and a variety of autoimmune processes against multiple organs including the hypophysis, eyes, thyroid, liver, pancreas and joints. In occasional cases, in the range of 5–20% of the patients, immune suppressive therapy is required and in rare cases it may lead to permanent damage [2, 3, 5, 7–11].

Both programs have proceeded to pivotal trials in patients with metastatic melanoma. A large phase II clinical trial of single agent ipilimumab in second line therapy concluded enrollment in January of 2007, while a similar clinical trial with CP-675,206 concluded enrollment in October 2006. Both clinical trials had response rate as primary endpoint, with the major difference in patient eligibility being the exclusion of patients with high LDH in the study with CP-675,206.

A large phase III clinical trial comparing DTIC with the combination of DTIC plus ipilimumab is currently enrolling patients. This clinical trial has as primary endpoint progression free survival. The phase III testing of CP-675,206 has the primary endpoint of overall survival, and compares single agent CP-675,206 with DTIC or temozolomide.

In conclusion, two monoclonal antibodies blocking CTLA4 have demonstrated ability to break tolerance to self-tissues and result in objective cancer regressions. Their clinical benefit in large pivotal clinical trials for the treatment of melanoma is currently being tested.

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S22. T-cell mediated melanoma treatment- how can it be developed

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Biological immunotherapy is represented by tumor Infiltrating Lymphocytes (TIL: i.e injection of T lymphocytes generated and selected ex-vivo from blood or from the tumor) which is adoptive immunotherapy. At the clinical level, previously it has been shown that T.I.L. were able to induce a regression of metastatic lesion (mean 30%) but without increase the overall survival (quick relapse). Recently five crucial points have been identified for the efficacy of this biological immunotherapy

1- *The tumour burden*

A strong interaction exists between T.I.L. used as adjuvant therapy in melanoma stage III (AJCC) and the number of invaded lymph nodes. Indeed, the injection of TIL in patients with only one invaded lymph node was associated with an increased relapse free survival and an overall survival.

2- *Migration of TIL to the metastatic stage*

Four papers, now with an immunological follow-up have shown the correlation between the therapeutic benefit and the survival and the preferential migration of specific T lymphocytes to the tumor sites.

3- *The specific TIL against melanoma antigens*

The therapeutic benefit is directly related to the percentage of tumor antigen specific T lymphocytes, against melanoma obtained in the expansion.

4- *The melanoma antigens*

Recent results strongly suggest that the infusion of reactive Melan-A/MART-1 specific lymphocytes was associated with clinical efficiency of TIL treatment. Furthermore, the amount of such lymphocytes seemed to be critical for patients bearing more than one invaded lymph nodes before the treatment.

5- *The mechanisms of escape to adoptive immunotherapy*

Three mechanisms could be decrease the efficacy of adoptive immunotherapy:

- T reg cells (CD4+ CD25+ Fox p3) presents in the final expansion injected to the patient

- Suppressive cytokines secreted in situ by tumor cells (IL-10, TGFβ….)
- Decrease expression of melanoma expression by tumor cells

S23. Cutaneous adverse events of new anti-cancer drugs

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The field of cancer therapy has been radically modified over the last few years by the use of new drugs targeting specific molecules involved in cancer initiation and/or progression.

Indeed, drugs targeting EGFR, VEGFR-1,-2,-3, BRAF and/or KIT, are now approved for the treatment of several cancers including colon, renal cell or lungs cancer as well as gastrointestinal stromal tumours and chronic myeloid leukaemia. For melanoma, drugs targeting VEGFRs, BRAF, MEK, CTLA-4 or PD-1 are being developed.

Although these targeted agents usually induce less severe side effects than classical chemotherapy, they are associated with a wide range of side effects, and cutaneous symptoms are often the most frequent.

Interestingly, in the case of EGFR inhibition, cutaneous toxicity seem to be correlated with tumour response.

The spectrum of skin manifestations observed with targeted agents is wide and some are not classically drug-associated symptoms like modification of hair pigmentation, paronychia, folliculitis, eyelashes trichomegaly, palm and sole keratodermia, keratoacanthomas…

For example, sorafenib, which is a novel orally active small molecule, multi-kinase inhibitor inhibiting wild type RAF genes products, V599-E mutant B-Raf, VEGFR-2, -3, PDGFR-β, Flt-3 and c-kit recently approved for renal cell cancer and presently tested in melanoma, is associated with skin modifications in about 90% of the patients : facial rash, hand-foot skin reaction, subungual haemorrhages, alopecia, epidermal cysts... Most of them are moderate and do not impact treatment continuation, however, in some patients, cutaneous side effects can impact quality of life and lead to treatment discontinuation.

This lecture will review the cutaneous effects of new targeted agents and suggest some pathophysiological hypotheses as well as symptomatic measures and treatments in order to prevent or limit the skin toxicity.

Close collaboration between oncologists and dermatologist is critical in order to carefully describe and evaluate cutaneous symptoms seen with new targeted agents because an accurate description is the first mandatory step toward studies investigating and assessing the pathophysiology of the observed symptoms. Early identification of these symptoms may allow for optimal preventive and curative symptomatic treatment when necessary. Further studies of these effects may lead to improved understanding of mechanisms underlying cutaneous events and skin physiology in general.

S24. Communication with the cancer patient

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Breaking bad news to a cancer patient is among the most challenging tasks of the daily work of an oncologist. However, training in communication skills for medical students and doctors is rare. Only few doctors were formally trained in communicating with their patients. Not only breaking bad news, but also communicating in emotionally burdened situations and answering difficult questions is a daily challenge of the practicing oncologist. What do we answer, when patients ask: "How long will I live with this cancer? What will be done, when the chemotherapy does not work? Please never tell my wife, how seriously ill I am ….!"? Physicians are trained to immediately give the correct medical answer when being asked a difficult question. However, in many situations in oncology with high emotional impact, correct medical answers do not exist or are not what the patient is asking for. The lecture will cover various communication skills which can be helpful in common situations in clinical oncology.

S25. Advances in the adjuvant therapy of melanoma

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No treatment evaluated for inoperable melanoma has ever shown any significant impact upon survival or durable remission benefit. For this reason, the evaluation of the most promising new immunobiological approaches in the adjuvant postoperative setting has become important. Systemic adjuvant therapy of melanoma with high-dose IFN has shown durable relapse free survival impact and in two randomized controlled trials has demonstrated significant survival benefit that has also been corroborated in meta analyses of studies across the globe. The benefit of adjuvant high-dose IFN has been linked with induction of autoimmune responses in trials conducted in the Hellenic Oncology Group and more recently corroborated in relation to the largest US Cooperative Group trial E1694. Multiparameter studies of pro-inflammatory cytokines performed in the context of the E1694 study have also identified pretreatment biomarkers of susceptibility to therapeutic benefit of high-dose IFNα that may allow more precise identification of patients that are likely to benefit from high-dose IFNα. Neoadjuvant evaluation of high-dose IFNα has identified molecular and immunological mechanisms of benefit that suggest rational further development of this effective modality with vaccines, anti-ganglioside antibodies and other potent immunomodulators such as anti-CTLA4 blocking antibodies. The careful evaluation of high-dose IFNα IV induction therapy that has been a component of all positive trials of the US Intergroup, is a high priority in the international trial E1697–both in terms of acquired tumor-induced mechanisms of immunosuppression and in terms of germline genetic determinants of the capacity to mount an immune response to tumor and other auto-antigens.

S26. First-line biochemotherapy and high-dose IL-2 in biochemotherapy failures in metastatic melanoma. The hospital Sirio-Libanes experience

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With the exception of the randomized phase III study conducted at MD Anderson Cancer Center, all other randomized phase III studies which compared biochemotherapy with chemotherapy alone have failed to show a significant impact on overall survival. Nonetheless, one cannot disregard the results from phase II studies conducted by physicians with extensive experience with IL-2-based therapies that have consistently shown that approximately 5 to 10% of patients with metastatic melanoma treated with biochemotherapy (BioCT) are long term disease-free survivors (LTDFS). Furthermore, the experience of the National Cancer Institute and the Extramural Study Group have also shown LTDFS in approximately 4% of the patients with metastatic melanoma treated with first line high-dose IL-2 therapy. Here we report our experience at Hospital Sirio-Libanes with BioCT as first line therapy and with HD IL-2 after progression on BioCT. We retrospectively reviewed all patients with stage IV melanoma with a follow-up of at least 6 months (mo) who received BioCT between 1/1998 -1/2007 under the supervision of one of the attending physicians of Hospital Sirio-Libanes. Patients with active metastasis to the central nervous system or poor renal, hepatic or bone marrow function were excluded. Treatment consisted of two 3-week-cycles of cisplatin, 25 mg/m2 IV D1-3, vinblastine, 2 mg/m2 IV D1-3, dacarbazine, 800 mg/m2 IV D1, IL-2 9 MIU/m2 IVCI D1-4 and interferon alpha-2b (IFN), 5 MIU SC D1-5 (slightly modified MD Anderson Cancer Center protocol). Responding patients received up to 4 additional cycles (for a maximum of 6), depending on tolerance. Metastasectomy was performed for residual disease at the discretion of the attending physician. A total of 65 pts (41 men, median age 46 years, median follow-up 63 months) received 227 cycles of BioCT (median 4 cycles/pt). No deaths occurred during treatment. To date, 56 pts (86%) have recurred and 49 (75%) have died. Median time to progression and overall survival were 6.7 and 12.3 mo, respectively. Response rates are as follows: CR 20%, PR 32%, for an overall response rate of 52%. LTDFS, defined as pt alive and recurrence-free

Table 1 Clinical characteristics of patients with LTDFS after BioCT alone

Patient #	1	2	3	4	5
Age (years)/Gender	51/M	47/M	33/M	43/F	33/F
Metastatic sites	Lung/skin	Lung/skin	Lung/liver/pancreas	Adrenal/lymph node	Skin and soft tissue
Previous treatment	No	No	CCNU, CDDP and tamoxifen	No	Adjuvant Carbo/DTIC
Number of cycles	3	3	6	6	6
Follow-up (mo)	29+	46+	60+	80+	113+

CDDP Cisplatin; *Carbo* carboplatin

at 24+ mo after BioCT, was observed in 8 of the 68 pts treated (29+, 46+, 60+, 66+, 71+, 80+, 96+, 113+ mo). 4 of the 8 pts were rendered disease-free by surgery following BioCT (3 PR and 1 SD; all with apparently viable melanoma at pathology). The remaining 4 pts achieved a CR and are LTDFS with BioCT alone (see Table 1 for clinical characteristics). Our experience demonstrates that LTDFS can be achieved in approximately 10% of pts with stage IV melanoma treated with first-line BioCT alone. Although small, this subset of pts justifies the use of BioCT for young, motivated and highly selected pts.

There are limited data on systemic therapy after progression following BioCT in patients with stage IV melanoma. High-dose IL-2 has a 16% overall response rate as first-line therapy, with approximately 4% of the pts achieving LTDFS. We retrospectively reviewed our experience with high-dose IL-2 as second line therapy in pts who had metastatic melanoma and had progressed during or after BioCT from 1/2000 to 1/2007. One treatment course consisted of IL-2, 600.000 IU/kg IVP q8h for a maximum of 14 doses per cycle, followed by an identical cycle after a one week of rest. Responding patients received 2 additional courses after 6 weeks of rest between courses. A total of 29 pts (18 male, 11 female); median age 46 (range 21–68) were treated with high-dose IL-2. Two patients received only 1 cycle of therapy due to rapid PD. One of the pts with rapid PD had treatment-related sepsis and was sedated instead of having the infection treated (considered treatment-related death). The remaining 27 patients received at least 1 full course (2 cycles) of therapy. Median number of cycles was 2 and median number of doses per cycle during cycles #1 and 2 were 10 and 9, respectively. Response rates using RECIST were as follows: CR, 1 pt (3.4%); PR, 7 pts (24%). Duration of response was 1, 3, 6+, 8, 9, 10, 12, and 18 months. There were no LTDFS in this cohort to date. Among the 9 patients with a previous response to BioCT, 1 pt had CR and 3 had PR with high-dose IL-2. Among the 6 pts whose disease previously progressed on BioCT, 3 pts achieved a PR. After a median follow-up of 50 months, median time to progression and overall survival were 2.5 and 7.8 mo, respectively. All pts were treated in a regular ward setting under the assistance of specialized and trained nurses on high-dose IL-2 therapy. High-dose IL-2 has antitumor activity after BioCT failure at least similar to the first line and can provide meaningful palliation in a small group of patients. Further studies such as gene profiling designed to better identify individuals who could potentially benefit most from BioCT and HD IL-2 are clearly warranted.

S27. A 65 CNTO95, a fully human monoclonal antibody that inhibits alphaV integrins, has antitumor and antiangiogenic activity in vivo

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AlphaV integrins are implicated in tumor-induced angiogenesis. CNTO95, a fully human antibody, recognizes and binds with highest affinity to alphaVbeta3 and alphaVbeta5. CNTO95 inhibited human melanoma cell adhesion, migration and invasion. Rat aortic ring-sprouting assays showed complete inhibition with CNTO95. A human melanoma xenograft model in nude mice CNTO95 inhibited tumor growth by 80%. In nude rat human xenograft models where CNTO95 binds and blocks tumor and host integrins, tumor weight was reduced by 99%. A dose escalating phase 1 trial in patients with solid tumors was conducted. Six subjects received extended therapy, one subject with angiosarcoma showed a PR of 9 months. A study in metastatic melanoma analyzing CNTO95 alone or in combination with DTIC is ongoing. Preliminary efficacy in the phase 1 dose-escalation part showed one CR and three SD. Preliminary safety analysis in phase 2 showed no concerns. Combinations and single agent treatment was well tolerated. CNTO95, has potent antitumor and antiangiogenic properties. CNTO95 is very well tolerated, no dose-limiting toxicities are observed, the maximum tolerated doses is not reached and it demonstrates clinical activity alone or in combination.