Mean sP-sel plasma levels (95% CI) before, after first bevacizumab application and after 6 weeks were 49.1 ng/ml (40.5−57.7), 40.3 ng/ml (32.5−48.2), (p = 0.0007) and 40.5 ng/ml (29.5−51.5), (p = 0.08).
Conclusions: Our data does not support the view that increased platelet activation or increased platelet adhesiveness and aggregation by bevacizumab is a relevant mechanism for thrombosis formation in the clinical practice. Mean sP-sel plasma levels were statistically significantly reduced by 17.9% after the first bevacizumab application. This may point to reduced platelet activation possibly contributing to the increased rate of haemorrhage associated with bevacizumab. However, this preliminary finding needs to be confirmed by additional investigations.

Melanoma and skin cancer

Oral presentations (Wed, 23 Sep, 09:00−10:30)
Melanoma and skin cancer

9300 ORAL

Expression alterations of genes located on the 7q31 region in human malignant melanomas

L. Vízkeleti[1], S. Ecsedi[1], Z. Rákosy[1], G. Emri[2], A. Bégány[2], A. Szöllosi[3], G. Méhes[4], R. Ádány[1], M. Balázs[1]. [1]*University of Debrecen Medical and Health Science Center, Department of Preventive Medicine, Debrecen, Hungary;* [2]*University of Debrecen Medical and Health Science Center, Department of Dermatology, Debrecen, Hungary;* [3]*University of Debrecen Medical and Health Science Center, Department of Physiology, Debrecen, Hungary;* [4]*University of Debrecen Medical and Health Science Center, Department of Pathology, Debrecen, Hungary*

FRA7G fragile site on 7q31.2-qter contains several genes affected in cancer development and progression. However, the role of 7q31 locus copy number alterations and the expression level of locus related genes in melanoma progression are slightly examined.
Based on previous aCGH results our aim was to simultaneously examine the copy number alterations of chr7 and 7q31 locus in 75 primary melanomas by FISH and correlate the genomic data with tumors' pathological parameters. The mRNA- and protein expression level of *cav1*, *met* and *tes* genes all located on this region was determined by QRT-PCR for 34 lesions and immunohistochemistry (tissue microarray) for 65 primary tumors.
The signal distribution by FISH was heterogeneous for both regions. Locus amplification was often detected in melanomas with metastasis formation, while lesions without metastasis showed rather locus deletion ($p < 0.05$; 5-year follow-up). Extra-copy of 7q31 was accompanied by chr7 polisomy ($p < 0.001$). All 3 genes were down-regulated in samples with ulceration, metastasis formation and >4mm thickness, which resulted in a more serious outcome. The co-presence of ulceration and metastasis strongly correlated with the changes in mRNA level of *tes* and *cav1* ($p = 0.01$ and $p = 0.003$, respectively). Interestingly, the more, than 2-fold decrease in *met* expression was seen only in samples with metastasis formation (8/12 specimens). This phenomenon did not depend on the 7q31 copies, but correlated with the *met* protein expression and usually accompanied by reduced *cav1* and/or *tes* expression level. There was a tendency that diminished *cav1*, *tes* and *met* mRNA level was associated with decreased expression of proteins. Therefore, primary melanomas with pathological signs of bad prognosis can be characterized with lower protein expression of these genes.
In conclusion, 7q31 amplification is resulted in a poor prognosis. Lower expression of the *met*, *tes* and *cav1* genes can contribute to an unfavorable outcome. The role of *cav1* and *tes* tumorsuppressor genes may be of greater importance on melanoma aggressivity, than the alterations of *met* oncogene. 7q31 copy number aberrations and the expression level of *met*, *cav1* and *tes* seem to be independent markers in human malignant melanomas. In the near future we plan to perform functional analysis on differently aggressive melanoma cell lines in order to determine the role of molecular pathways and their relationships connected to these proteins in melanoma progression.

9301 ORAL

Identification and characterization of cancer stem cells in melanoma

Y. Welte[1], J. Adjaye[1], E.F. Vencio[1], B. Timmermann[1], H. Lehrach[1], C.R.A. Regenbrecht[1]. [1]*Max-Planck Institute for Molecular Genetics, Department Lehrach, Berlin, Germany*

The identification of cancer stem cells in various malignancies led to the hypotheses that these cells have exclusive ability of self-renew, contribute to the plasticity of the tumours and may be the cause for failures of cancer therapies. Several markers of melanoma stem cells have been described in recent studies but further investigations are necessary to identify, better define, and understand origin and function of cancer stem cells. If confirmed, therapeutic strategies may need to be redirected towards these cells to circumvent the failure of conventional therapies.
Using three different approaches we investigated ten low passage melanoma cell lines established from metastatic lesions of melanoma patients for the existence of putative cancer stem cells. The results of these approaches, i.e. the enrichment of cancer stem cells in embryonic stem cell medium containing FGF2, the identification of cancer stem cells as side population via staining with the DNA-binding dye Hoechst 33342 and the analysis of melanoma cell lines for the expression of known stem cell and cancer stem cell markers, suggest that there is not a single method so far known that allows to specifically depict all cancer stem cells in melanoma. Therefore we then focused on the key stem cell properties like the ability to self-renew to find further common characteristics between stem cells and cancer stem cells. Thereby, we found pathways like the FGF signaling cascade only active in melanoma cells cultivated in embryonic stem cell medium or in sorted cancer stem cells after normal culture conditions. Furthermore, the self renewal factor OCT4 is only expressed in cancer stem cells but not in non-cancer stem cells.
Finally, we compared cancer stem cells and bulk tumor cells by using a RNA-sequencing approach on the Illumina platform to identify activated and deactivated oncogenes and signaling pathways that allow exclusive identification and targeting of cancer stem cells.
There is good evidence supporting a shift of paradigms in understanding cancer, but still the origin of cancer stem cells and their defining properties remain elusive. Only by combining approaches from stem cell and cancer research, it may become possible to identify, characterize and use these cells in future cancer treatment.

9302 ORAL

Excellent long-term survival of patients with minimal sentinel node tumor burden (<0.1 mm) according to Rotterdam Criteria: a study of the EORTC melanoma group

A.C.J. van Akkooi[1], P. Rutkowski[2], I.M. van der Ploeg[3], C. Voit[4], C. Robert[5], H.J. Hoekstra[6], O.E. Nieweg[3], Z.I. Nowecki[2], A. Spatz[5], A.M.M. Eggermont[1]. [1]*Erasmus University Medical Center - Daniel den Hoed Cancer Center, Department of Surgical Oncology, Rotterdam, The Netherlands;* [2]*M. Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Department of Soft Tissue/Bone Sarcoma, Warsaw, Poland;* [3]*the Netherlands Cancer Institute / Anthoni van Leeuwenhoek Hospital, Department of Surgery, Amsterdam, The Netherlands;* [4]*Charité Humboldt University, Department of Dermatology, Berlin, Germany;* [5]*Institut Gustave Roussy, Department of Pathology, Villejuif, France;* [6]*University Medical Center Groningen, Department of Surgical Oncology, Groningen, The Netherlands*

Background: Many studies have identified Sentinel Node (SN) tumor burden as a prognostic factor for additional non-SN (NSN) positivity and / or disease-free (DFS) and melanoma specific survival (MSS). It remains unclear if pts with minimal SN tumor burden can safely be managed without Completion Lymph Node Dissection (CLND). Pts with minimal SN tumor burden might be at risk for late recurrences (> 5 years).
Methods: Slides of 663 SN positive patients were reviewed for this pan-European study collaboration in 6 major centers. Slides were reviewed for the microanatomic location and SN tumor burden according to the Rotterdam Criteria (<0.1 mm, 0.1−1.0 mm and >1.0 mm) for the maximum diameter of the largest metastasis. MSS, DFS and distant metastasis-free survival (DMFS) rates were calculated, as was NSN positivity.
Results: In 663 SN positive pts, the mean and median Breslow thickness was 4.6 and 3.3 mm. Ulceration was present in 50% of melanomas. 73 pts had metastases <0.1 mm (11%), 260 pts (39%) had 0.1−1.0 mm metastases and 330 pts had metastases > 1.0 mm (50%). Mean and median follow-up was 47 and 38 months for all patients (range 1−172). Patients with metastases <0.1 mm had mean and median follow-up of 59 and 56 months, 47% (34pts) had follow up > 5 years and 25% (18 pts) had follow-up longer than 74 months (range 3−132).
5-year MSS rates were 93% for metastases <0.1 mm, 71% for 0.1−1.0 mm and 57% for > 1.0 mm ($p < 0.001$). Estimated 10-year rates were 93% for

<0.1 metastases, 58% for 0.1−1.0 metastases and 40% for metastases > 1.0 mm. 5-year DMFS rate was 91% for metastases <0.1 mm. NSN positivity occurred in 6% of <0.1 mm, 13% of > 0.2 mm metastases, 16% of 0.1−1.0 and 28% of metastases > 1.0 mm ($p < 0.001$).
Conclusion: This large multicenter experience (n = 663) has demonstrated that long-term follow-up of melanoma patients with minimal SN tumor burden (<0.1 mm) indicates very low relapse rates and excellent MSS, seemingly identical to SN negative patients. With prolonged follow-up, an increase in the occurrence of relapses of any kind between 5 and 10 years follow up has not been identified, and excellent 10-year survival rates are expected.

9303 ORAL

New ultrasound morphology criteria can predict melanoma metastases in the sentinel lymph node (SN) and correlate with tumour burden and survival

C. Voit[1], A. Akkooi van[2], G. Schaefer-Hesterberg[1], A. Schoengen[3], W. Sterry[1], A.M.M. Eggermont[2]. *[1]Charité Campus Mitte/Campus Virchow Klinikum, Dermatology, Berlin, Germany; [2]Erasmus University Medical Center, Daniel den Hoed Cancer Center, Rotterdam,Tthe Netherlands, Department of Surgical Oncology, Rotterdam, The Netherlands; [3]Department of Medical Oncology Armed Forces Hospital Ulm Germany, Medical Oncology, Ulm, Germany*

Background: We demonstrated that US guided FNAC (fine needle aspiration cytology) prior to SN biopsy can identify up to 65% of SN-positive patients (EJC, 2007; 5(6):11: abstract 3BA). We presented for the first time US patterns of SN-involvement at ASCO 2008 (JCO 26: 2008 (suppl; abstr 9014). The aim of the present study is to show in how far these patterns correlate with progression of disease, tumor burden, survival and prognosis.
Methods: Prior to SN-biopsy patients (pts) underwent lymphoscintigraphy followed by US-exam. US images were prospectively scored for 6 morphologic criteria: presence of peripheral perfusion, loss of central echoes, balloon shaped lymph node, moreover for hump structure, echo-poor islands, cap structure. FNAC was performed in suspicious US. All pts underwent a SN biopsy. Final SN pathology was the gold standard. Sensitivity, specificity and negative/positive predictive value (NPV and PPV) of combinations of US patterns were calculated and correlated with tumor burden and survival. Hazard ratios (HR) were calculated for the patterns by multivariate analysis.
Results: Since 2001 850 consecutive pts have been included into a prospective database. Median Breslow thickness of the first 400 stage I/II melanoma pts was 1.8mm, median follow-up 42 months. Balloon Shape (BS) & Loss of Central Echoes (LCE) are often linked (up to 83%) and are *late* signs correlating with high tumor load. In contrast the presence of Peripheral Perfusion (PP) is an *early* sign, correlating with small tumor load. PP and/or BS and/or LCE together raise the sensitivity of US alone to > 80%, spec.80%, PPV 52%, NPV of 94% ($p < 0.001$). Overall Survival of neither vs. Peripheral Perfusion (PP) only vs. BS/LCE (with or without PP) was 93% vs. 87% vs. 56% and Distant Metastasis-Free Survival was 74% vs. 60% vs. 26%. BS/LCE was a late sign correlating with high tumor load, fast progression and a high HR (5.50). PP alone was an early sign correlating with small tumor load, slow progression and a low HR (2.19).
Conclusions: We have identified 2 ultrasound morphology signs of lymph node metastasis in melanoma patients: Peripheral perfusion as early and Balloon Shaped Lymph Node and / or Loss of Central Echoes as late signs. BS and/or LCE indicate high tumor load, PP alone indicates small tumor load in the SN. With these criteria we can identify any amount of SN tumor burden correctly prior to the surgical SN procedure in 75% - 90% of cases. Balloon Shaped Lymph Node and/or Loss of Central Echoes and Peripheral Perfusion are independent prognostic factors for Survival.

9304 ORAL

Identification of tumor biopsy markers as potential predictors of ipilimumab clinical activity in patients with advanced melanoma

H. Schmidt[1], O. Hamid[2], A. Nissan[3], M. Guida[4], S. Aamdal[5], J. Hansson[6], R. Ridolfi[7], D. Berman[8], S.D. Chasalow[9]. *[1]Aarhus University Hospital, Oncology, Aarhus, Denmark; [2]The Angeles Clinic and Research Institute, Neuro-Oncology, Santa Monica, USA; [3]Hadassah University Hospital, Surgery, Jerusalem, Israel; [4]National Institute of Cancer, Medical and Experimental Oncology, Bari, Italy; [5]Norwegian Radium Hospital, Clinical Cancer Research, Oslo, Norway; [6]Karolinska Institutet, Oncology-Pathology, Stockholm, Sweden; [7]IRST Cancer Institute, Immunotherapy and Cell Therapy, Meldola, Italy; [8]Bristol-Myers Squibb, Global Clinical Research, Princeton, USA; [9]Bristol-Myers Squibb, Global Biometrics and Science, Pennington, USA*

Background: Ipilimumab, a monoclonal antibody that blocks cytotoxic T-lymphocyte antigen-4, has demonstrated activity in advanced melanoma patients (pts). As part of a completed Phase II study (CA184−004), we sought to identify tumor biomarkers of early ipilimumab effects that can be used to predict clinical activity.
Methods: Pts received ipilimumab at 3 mg/kg (n = 40) or 10 mg/kg (n = 42), given every 3 weeks (Q3W) ×4; eligible pts could receive ipilimumab Q12W starting at Wk24. Ninety-one fresh tumor biopsy samples (50 pre-treatment and 41 post-treatment at Wk4) from 57 pts were evaluable by immunohistochemistry (IHC) and hematoxylin and eosin (H&E) staining. The expression of 8 proteins, including FoxP3 and indoleamine 2,3-dioxygenase (IDO), was assessed by IHC. Six tumor characteristics, including tumor-infiltrating lymphocytes (TILs), were assessed by H&E; mRNA expression levels were quantified in biopsy sub-samples by Affymetrix microarray analysis (54 pts with both pre- and post-treatment data). Response was evaluated using modified World Health Organization criteria.
Results: Clinical activity (complete response, partial response, or stable disease ⩾24 wks from first dose) was associated with increased baseline expression of FoxP3 (n = 33) and IDO (n = 35), and with an increase in TILs at Wk4 relative to baseline (n = 27) [Table]. In tumor biopsies, expression of 466 mRNA probe sets had a significant change from baseline (after multiplicity correction, q-value <0.05). Genes with significant increased expression included various immune-response genes, e.g., immunoglobulins, granzyme B, and T cell receptor alpha and beta subunits. Genes with significant decreased expression included known melanoma antigens, e.g., tyrosinase-related protein 2, gp100, and melan-A.

Biomarker[a]	Clinical activity	No clinical activity	P value[b]	Dose response
TILs at Wk4, change from baseline	4/7 had increase 0/7 had decrease 3/7 had no change	2/20 had increase 3/20 had decrease 15/20 had no change	P = 0.005	No
FoxP3 expression at baseline	6/8 were positive	9/25 were positive	P = 0.014	N/A
IDO expression at baseline	3/8 were positive	3/27 were positive	P = 0.012	N/A

[a]Using 3-point scale for TILs (absent, ⩽50%, >50%) and a 9-point scale for IHC (0−4, in 0.5 increments); [b]P-values were not corrected for multiple testing.

Conclusions: Increased baseline expression of tumor FoxP3 and IDO, and increase from baseline of TILs at Wk4, may be used to identify pts who will experience clinical activity with ipilimumab.

9305 ORAL

Activity of sunitinib in advanced malignant melanoma and its correlation with potential predictive biomarkers

L. Decoster[1], I. Vande Broek[2], D. Declerq[1], E. Anckaert[3], F. Majois[4], J.F. Baurain[5], H. Denys[6], J. De Mey[7], B. Neyns[1], J. De Grève[1]. *[1]UZ Brussel, Medical Oncology, Brussels, Belgium; [2]UZ Brussel, Clinical Haematology, Brussels, Belgium; [3]UZ Brussel, Laboratory of Clinical Chemistry and Radio-Immunology, Brussels, Belgium; [4]CHU Jolimont, Medical Oncology, Brussels, Belgium; [5]Clinique Saint Luc, Medical Oncology, Brussels, Belgium; [6]UZ Gent, Medical Oncology, Brussels, Belgium; [7]UZ Brussel, Radiology, Brussels, Belgium*

Background: Sunitinib is approved for the treatment of renal cell carcinoma and GIST tumours. It is a small molecule that inhibits members of the split-kinase domain family of tyrosine kinase receptors, including VEGFR, PDGFR, c-KIT and RET kinases. These kinases are important for neoangiogenesis, tumor cell proliferation and survival. Treatment options for advanced melanoma after dacarbazine-based chemotherapy are limited. We report here our initial observations with sunitinib in advanced melanoma patients, whose disease failed at least one line chemotherapy.
Methods: Patients with locally advanced or metastatic melanoma, whose disease failed at least one line of dacarbazine-based chemotherapy were