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

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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

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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

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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