a significant control of CINV in pts receiving moderately/highly emetogenic chemotherapy. We prospectively evaluated the efficacy of a single or double i.v. dose PALO in pts undergoing HDT and ASCT.
**Methods:** A total of 60 pts (M/F = 32/28), median age 45 yrs (r16−64), with diagnosis of lymphoma (29), myeloma (24), sarcoma (5), acute leukemia (1), breast cancer (1) were accrued. The first cohort (30 pts) received a single iv PALO dose (0.25 mg) plus 8 mg of dexamethasone (DMS) 30′ before starting of HDT while in the second cohort (30 pts) the first dose was followed by a further PALO (0.25 mg)/DMS (8 mg) injection 48h after HDT. The distribution of conditioning regimens (high-dose melphalan = 28, BEAM = 25, MitoMel = 6, ThioEpiCTX = 1) was comparable between the two cohorts. Acute (24h) and delayed (120h) CINV episodes were rated by the visual analogic scale (MASCC/MAT) while CINV impact on daily activities was self-assessed by pts (at 120h from starting of HDT), through the Functional Living Index-Emesis (FLIE) tool.
**Results:** No significant differences between the two groups (single vs double PALO) emerged as to acute CINV evaluation (MAT) since 98% of pts achieved a complete response (CR = no emesis, no need for rescue therapy) with only 17 pts (28%) experiencing moderate nausea (median intensity = 5, r 1−10). Double-dose PALO displayed a trend for a better control of delayed nausea which occurred in 53% vs 77% of pts ($p = 0.0581$). In addition, double PALO dosing had a highly significant impact on nausea-related modifications of daily activities. FLIE nausea score was of a median value of 55.26 (r47.5−58.9) in pts receiving two doses of PALO vs 40.92 (r35−45.2) for pts treated with the single PALO dosing ($p = 0.0009$).
**Conclusion:** Our results indicate that double dose PALO achieves an optimal control of acute/delayed CINV and significantly reduces the detrimental impact of nausea on daily activities in patients undergoing HDT. The impressive activity of PALO in the ASCT setting might be possibly improved by combination with NK1 receptor antagonists.

**9250** POSTER

**Secondary malignancies after stems cell transplantation – a single centre experience**

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**Background:** It is considered that patients (pts) submitted to Stem Cell Transplantation (SCT) are at increased risk of develop secondary primary malignancies which may be related to several factors, such as primary malignant disease, radiation/chemotherapy treatment or graft-versus-host disease. Authors aim to report a single centre experience on this issue.
**Material and Methods:** Retrospective analysis of pts who developed secondary malignancies (SM), selected from all stem cell transplanted patient, from June 1989 to December 2008, in the Portuguese Oncology Institute, Porto. Statistical analysis was performed using SPSS v16.0.
**Results:** A total of 1026 patients underwent 1190 SCT during this period - 599 autologous (AutoSCT) and 591 allogenic (AlloSCT). SM after SCT were identified in 28 pts (2.7%) – 16 in autoSCT and 12 in alloSCT. 78.6% of pts were male and median age was 38 years (range: 3−66). Conditioning plan did not predict total body irradiation or any other radiation strategy. Most common primary malignancy was hodgkin lymphoma (7 cases), followed by non-hodgkin lymphoma and acute myeloid leukaemia (6 cases each). Secondary malignancies, according to their frequency, were: oral cavity/tong, colon, sarcoma, acute myeloid leukaemia, and myelo-displasic syndrome (3 cases each); thyroid, urinary tract and cervix (2 cases each); breast, esophagus, PNET, skin (basocelular carcinoma), renal, gastric and hepatocelular cancer (1 case each). Four pts developed a third malignancy (sarcoma, esophagus, thyroid and non-hodgkin lymphoma). SM was responsible for 12 (42.9%) of the 16 deaths observed in this set of patients. Ten years overall survival after SM diagnose was 41.7% (31.2% in AutoSTC and 60% in AlloSCT). Median time from STC to SM was 51.5 months (range: 1−177). Hodgkin lymphoma seems to be related with secondary myelo-displasic syndrome ($p = 0.055$). No statistical significant independent prognostic factor was found.
**Conclusions:** A low incidence of SM was found, particularly in AlloSCT pts. It is important to conduct larger studies in order to determinate possible risk factors and better manage these events.

**9251** POSTER

**Hepatitis B virus (HBV) reactivation in patients with resolved prior HBV according to HBV profiles in the era of immunochemotherapy**

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**Background:** The epidemiology and factors affecting HBV reactivation in patients with resolved prior HBV (HBsAg -ve; anti-HBc +ve) has not been defined. We aim to analyse the HBV profiles of patients with resolved prior HBV and correlate them with the risk of reactivation, particularly in those receiving immunochemotherapy.
**Materials and Methods:** 374 patients from 2005−09 were included. HBsAg, anti-HBc, anti-HBs and HBV DNA were tested. HBV reactivation was defined as the reappearance of HBsAg with an increase in HBV DNA levels when compared with baseline.
**Results:** 374 had both HBsAg and anti-HBc tested, 124 (33.2%) had resolved prior HBV. The proportion of resolved HBV increases with age, rising from 12.5% in patients 10−20 yrs to 58.5% in 70−80 yrs, ($p < 0.01$). 95 were tested for anti-HBs, which was positive in 67 (67.4%). 87 were tested for HBV DNA, which was detectable in 5 [(5.7%), 2 were anti-HBs positive, 2 were negative and 1 was not tested]. Overall, 103 patients with prior HBV received systemic treatment and 3 reactivated (2.9%). Of note, 1 reactivated despite having high anti-HBs titer (90mIU/ml). Among patients receiving Rituximab-based treatment and no anti-viral prophylaxis, the reactivation rate was 3.1% (2/65). Of note, 4 patients received maintenance-Rituximab and 1 (25%) reactivated. Of the 5 patients with detectable HBV DNA, 1 reactivated (20%).
**Conclusions:**
1. In 3 lymphoma patients in an endemic area have prior HBV, although the prevalence appears decreasing in younger patients, possibly due to an active immunization program;
2. 6% of our patients were viraemic despite being HBsAg negative, suggesting that a polyclonal EIA (enzyme immunoassay) for HBsAg may have a role in detecting HBV mutants, compared to the current monoclonal EIA;
3. The rate of reactivation in patients with resolved HBV is relatively low, even among patients treated immunotherapy without antiviral prophylaxis. This coupled with the high prevalence of resolved HBV suggest that routine prophylaxis may not be feasible or indicated in all;
4. Nonetheless, our data caution that patients receiving maintenance-Rituximab or having detectable HBV DNA may be at increased risk of reactivation and the role of prophylaxis further evaluated;
5. Anti-HBs appears to have a limited immune-protective role as 2 patients were viraemic despite having high anti-HBs titers (values). Further, 1 patient without viraemia reactivated despite having high anti-HBs titer;
6. These results are novel.

**9252** POSTER

**Influence of bevacizumab on platelet function in vivo**

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**Background:** Haemorrhage, as well as arterial and venous thromboembolism are well known side effects associated with bevacizumab. The pathophysiological mechanisms involved are poorly understood. We hypothesized from preclinical data, that platelet function may be a possible site of interaction of vascular endothelial growth factor (VEGF) and bevacizumab. We aimed at testing this hypothesis in a clinical observational study.
**Material and Methods:** Platelet adhesive and aggregatory functions were tested with a platelet function analyzer (PFA-100) inducing platelet activation with either epinephrine (EPI) or adenosinediphoshpate (ADP). Soluble P-selectin (sP-sel) as a marker of platelet activation was measured using a commercially available immunoassay-kit. In 22 patients with advanced metastatic disease treated with cytostatic chemotherapy and bevacizumab, PFA-100 closure times (CTs) and sP-sel plasma levels were assessed before and immediately after first treatment with bevacizumab and after 6 weeks of treatment.
**Results:** Mean PFA-100 CTs (with 95% confidence interval (CI)) before, after first bevacizumab application and after 6 weeks were 112.6 sec (99.8−125.4), 110.9 sec (98.5−123.3) and 110.8 sec (94.0−127.7) respectively with EPI ($p > 0.05$). CTs for ADP were 79.7 sec (71.9−86.7), 82.1 sec (72.7−90.8) and 81.0 sec (72.8−89.2), respectively ($p > 0.05$).

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**

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**

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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