olites of the mevalonate pathway which trigger proliferation of a subset of cytotoxic gamma, delta T cells and cytokine release. Several studies are now in progress investigating whether this immunomodulatory effect of zoledronic acid can be utilized in oncology to enhance its therapeutic potential beyond the well established inhibition of tumour-induced osteolysis.

S47. BONE SIALOPROTEIN IS PREDICTIVE OF BONE METASTASES IN RESECTABLE NON SMALL CELL LUNG CARCINOMA: A CASE–CONTROL STUDY AND PREVALENCE DATA

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Background: Non small cell lung cancer (NSCLC) is the leading cause of cancer related deaths, mostly secondary to diffuse extra-thoracic spread of the disease in several organs and systems. Bone metastases (BM) may be present at diagnosis or develop in the follow up, are associated with a worse prognosis, and currently there are no chemical or biological markers predicting their clinical onset. Several molecules are potential factors favouring bone dissemination by cancer cells, including cell cycle proteins, angiogenetic factors, extra-cellular matrix proteins and their inhibitors, serum and plasma proteins implicated in bone resorption mechanisms. Increased levels of some of these molecules (periostin, BSP and osteopontin) were found in colon, breast and prostate cancers. Their role in lung cancer is controversial. Aim of this study was to investigate the predictive and prognostic value of bone resorption-related molecules in favouring or modulating the colonisation of bone tissue during haematogenous spread of NSCLC.
Methods: Thirty cases of resected NSCLC which developed BM (group A – mean follow up time 27.2 months) were matched for several clinico-pathological parameters (including age, sex, stage of the disease, histology, differentiation grade, adjuvant therapy) to 30 cases of resected NSCLC without any metastases (group B – mean follow up time 75.1 months) and 26 resected NSCLC with non-bone metastases (group C – mean follow up time 21.1 months). Primary tumor samples were investigated by a standard automated immunoperoxidase procedure for 10 markers previously recognized to be involved in bone resorption or metastatization process (cathepsin K, bone sialoprotein [BSP],VEGF, MMP-2, p53, RECK, TIMP-1, CD-117, Ki-67 and TRAcP). For statistical analysis, the staining distribution in tumor cells was assessed by a semi-quantitative score (0, <10%, 10–50%, >50% positive tumor cells). Differences among groups were estimated by χ^2 test, whereas the prognostic impact of clinico-pathological parameters and marker expression was evaluated by univariate and multivariate analyses. An additional series of 120 resected consecutive NSCLC was also tested for BSP expression prevalence (group D).
Results: Among the different markers investigated, BSP expression was significantly higher in bone metastatic cases (80%) compared to 20% and 31% of groups B (non metastatic) and C (non-bone metastases), respectively ($p < 0.001$). BSP expression did not show any difference according to tumor histotype or

any other characteristics. In addition, taking all the three groups together, or the metastatic groups (groups A and C) alone, BSP expression was also shown to be related to poor outcome ($p = 0.02$ by Mantel–Cox test). None of the other markers was differentially expressed within the groups or demonstrated a prognostic impact, both in terms of overall survival and of time interval to metastases. BSP was further estimated in 120 resected NSCLCs (M:F ratio 3:1; mean age 67 years; histotype: adenocarcinomas 55%, squamous cell carcinoma 39%, others 6%; stages: I 54%, II 17%, III 29%) and a prevalence of 40% observed, without any statistically significant difference according to histotype or other clinico-pathological parameters.
Discussion: In this study, we have shown that BSP is significantly more expressed in a series of NSCLC metastatic to bone as compared with matched control groups of NSCLC (metastatic or non metastatic) which did not progress to bone in the same period of time. BSP expression was also found to be predictive of poor prognosis, but not related to the time interval to distant spread. Moreover, in a large consecutive series of resected NSCLC we observed a prevalence of BSP protein expression of 40%. Interestingly, this percentage of positivity is intermediate between that in group A on the one side, and groups B and C on the other. The biological significance of BSP expression in tumors progressing to bone metastases is not fully understood. The balance of bone apposition and resorption involves several molecules, locally produced or possibly blood-born, which act through different specific circuits. BSP itself may be powering the effect of bone resorption and facilitate bone colonisation by tumor cells. In in vitro models, BSP favoured cancer cell invasiveness through a linkage with integrins and MMP2. Inhibition of BSP-MMP2 complex was able to block BSP-enhanced invasiveness. Our findings suggest that in the future NSCLC patients with BSP expression, may benefit of BSP inhibitors and may also be reasonably good candidates for preventive treatments (i.e., bone metabolic agents) in order to block, reduce or delay the osteotropism of cancer cells. In conclusion, immunohistochemical expression of BSP in resected NSCLC strongly predicts bone dissemination, and may therefore be useful in selecting patients for treatments targeted to contrast bone metastatic spread.

S48. DIFFERENT ROLES OF "STEM CELLS" IN GLIOMAS

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CD133 positive "Cancer Stem Cells" (CSC) have been shown to initiate and maintain glioblastoma growth. The first aim of our studies was to further characterize CD133+ cells in gliomas of different grades with respect to their prospective origin and differentiation potential. CD133+ cells could be identified in gliomas grade II–IV. Co-expression of CD133 and Musashi-1 indicated a neural stem cell character of CD133+ cells. Expression of both markers was clearly grade dependent with up to 20% of cells being CD133+ in GBM. Under different culture conditions, CD133+ cells isolated from gliomas lost CD133 expression and started expression of