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.

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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 extrathoracic 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 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 semiquantitative 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.

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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

glial, neuronal and endothelial markers demonstrating pluripotency of differentiation potential. These findings point towards an essential role of CD133/Musashi-1+ cells in glioma biology making these cells a potential target for future therapies. On the other hand, stem cells are currently evaluated as potential carriers of anti-glioma therapies. Therefore, the second aim of our studies was to assess intracerebral distribution patterns of mesenchymal stroma cells (MSC) after local versus systemic application. Human MSC (hMSC) were isolated from bone marrow biopsies carried out for haematological indications. U373-GFP gliomas were generated by orthotopic implantation. After local application of hMSC, migration of hMSC towards the tumor was observed. In a second setting, intravenously administered MSC transfected with a RFP/Tie-2 promotor gene showed extensive tropism to the glioma. RFP expression indicated integration of MSC into the intratumoral vasculature. Therefore, MSC might be valuable candidates as carriers for an anti-tumor, especially an anti-vascular gene therapy. Altogether, stem cells might play roles in the "cause" as well as in the "cure" of glioma.

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S49. TARGETED DELIVERY OF CHEMOTHERAPY USING MICROENCAPSULATED CELLS FOR GDEPT

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Some of the most potent cytotoxic drugs, e.g. ifosfamide, are hampered in their effectiveness in certain cancers due to severe side effects in elder patients. A way to circumvent this problem is to employ gene directed enzyme prodrug therapy by delivering a second site of drug activation at the tumor site. This concept was developed using ifosfamide as the prodrug and the metabolising cytochrome P450 subenzyme CYP2B1 for conversion. The gene was transfected in 293 cells. To protect the genetically modified cells from the host immune system and, conversely, to protect the host from the allogenic cells, those were microencapsulated in sodium cellulose (diameter $\approx$ 0.8 mm). In an experimental setting, the microcapsules containing CYP2B1 expressing cells were directly injected in pre-established human pancreatic adenocarcinomas on nude mice. After treatment with low-dose ifosfamide, 20% CR was achieved. Identical results were obtained in a syngenic rat pancreatic carcinoma model. Further, this experimental approach has very successfully been applied to experimental peritoneal carcinomatosis as well as naturally occurring breast carcinomas in dogs. For clinical use, a feasibility study in pigs demonstrated the safety of agiographic intra-arterial placement into a pancreatic artery. After completion of IRB and registration with authorities, a clinical phase I/II study in patients with inoperable pancreatic adenocarcinoma was performed. The concept proved to be safe. 2/14 patients demonstrated a partial remission, the reminder was stable. Mean OS was 44 weeks, one year survival 32%.

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S50. DEVELOPING STRATEGIES FOR TUMOR VACCINATION

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Vaccination against cancer has had a variable history, with claims of success often fading into disappointment. The reasons for this include poor vaccine design, inadequate understanding of the nature of the immune response, and a lack of objective measures to evaluate performance. The characterization of tumor-associated antigens (TAAs) recognized by human T lymphocytes in a MHC-restricted fashion has opened new possibilities for specific vaccine approaches to the treatment of human cancers. Recent findings include vaccine formulation, relevant knowledge concerning mechanisms of induction of effective immunity from preclinical models, and translation into clinical trials. We now have novel vaccine strategies to activate specific attack on tumor cells and we understand more about activation and regulation of immunity against cancer (co-stimulation versus co-inhibition, regulatory T cells). We also have modern assays using surrogate markers (MHC multimer analysis, IFN-γ Elispot assay) to correlate with clinical effects. Although early clinical vaccine trials based on synthetic peptides, proteins, 'naked' DNA, tumor-RNA, dendritic cells, and recombinant vaccinia viruses indicate that vaccines can induce immune responses and tumor regression in some cancer patients, careful study design and use of standardized clinical and immunological criteria are needed. Basic principles of tumor vaccination and clinical trials will be discussed.

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S51. MOLECULAR STAGING OF THYROID CANCERS AND ASSOCIATED FAMILIAL SYNDROMES – CONSEQUENCES FOR SCREENING AND THERAPY

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Disseminated tumor cells in tissue, blood or bone marrow samples of patients with thyroid carcinoma are detectable by PCR assays by using different molecular tumor markers. The aim of our study was to correlate the results of molecular staging with the patients' follow-up.

Patients and Methods: Eighty-seven tumor, 43 blood and 14 bone marrow samples of patients with thyroid carcinomas were obtained during surgery and subjected to Cytokeratin 20 (CK20) and PreproGastrin-releasing peptide (PreproGRP) PCR systems.

Results: An expression of CK20 transcripts was detected in all of the medullary thyroid carcinomas (MTC), 63% of follicular thyroid carcinomas (FTC), 43% of papillary (PTC) and 17% of anaplastic (ATC) carcinomas . In FTC and PTC an expression of CK20 was seen in 54% in primary tumors and in 42% in soft tissue or lymph node recurrence. 75% of the patients with CK20-positive FTC were disease-free at follow-up compared to none of the patients with CK20-negative FTC. PreproGRP was found in 100% of MTC tissue samples. Overall, disseminated tumor cells of CK20-positive carcinomas were detected in 33% of the blood samples. In MTC,