Therefore, EGFR signalling in colon cancer is modulated at the level of receptor capacity, receptor heterodimerization, regulation of intracellular tyrosine kinase activity, ligand presentation and by other costimulatory receptor pathways. These mechanisms have to be considered when therapeutic strategies using EGFR-inhibition are evaluated.

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S45. THERAPEUTIC DECISIONS FROM MOLECULAR STAGING IN COLORECTAL CANCER

Jürgen Weitz. *Department of Surgery, University of Heidelberg, Germany.*

Colorectal cancer is one of the most prevalent cancers worldwide. Whereas surgery is the basis of therapy, chemo- and/or radiotherapy are used frequently to reduce local and distant recurrences. Many prognostic factors have been defined, with only the UICC classification being relevant for therapeutic decisions at present. The prognosis for an individual patient, however, still cannot be sufficiently predicted. Therefore, many patients will receive radio-/chemotherapy that do not benefit from this treatment. Other patients might not receive radio-/chemotherapy as they are judged to be at a low risk for recurrence, but will develop recurrent disease. Better prognostic factors are therefore needed, in order to individualize the therapeutic strategy. As recurrences after complete tumor removal are most likely caused by disseminated tumor cells, it seems to be a logical approach to develop methods to detect these cells. Ideally, patients in whom tumor cells can be detected should have a worse prognosis and should therefore benefit from adjuvant therapeutic strategies. The effect of this therapy should then become apparent by a reduced detection rate of disseminated tumor cells. Even though some studies demonstrate a prognostic relevance of disseminated tumor cells, the prognostic relevance of these cells is not generally accepted. Due to the heterogeneity of disseminated tumor cells, demonstration of the mere presence of these cells will most likely not be an adequate basis for therapeutic decisions. Molecular characterization of disseminated tumor cells and/or of the primary tumor might be a more successful approach in this respect. The clinical relevance of molecular staging for therapeutic decisions, however, has still to be proven in well designed clinical trials.

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S46. ANTI-TUMOUR POTENTIAL OF ZOLEDRONIC ACID (Zometa®)

Jonathan Green. *Novartis Institutes for BioMedical Research, Basel, Switzerland.*

Bisphosphonates have been used extensively for more than 3 decades to inhibit osteoclastic activity in a variety of benign and malignant diseases characterized by increased bone resorption. Due to the bisphosphonate moiety, these compounds bind avidly to bone mineral and accumulate at sites of active bone turnover. During bone resorption they are released and ingested by osteoclasts via fluid-phase endocytosis. The nitrogen-containing bisphosphonates (N-BPs) inhibit a key intracellular enzyme in the mevalonate pathway, farnesyl pyrophosphate synthase, thereby reducing prenylation of small GTPase signalling proteins that are essential for osteoclast function and survival. Consequently, bone resorption is impaired and osteoclast apoptosis is induced.

Zoledronic acid (Zometa®) is a novel compound which retains the bisphosphonate "bone hook" and possesses a heterocyclic imidazole substituent containing 2 nitrogen atoms. X-ray crystallography studies with human farnesyl pyrophosphate synthase have shown that the zoledronic acid molecule binds with high affinity to the active site of the enzyme and induces an irreversible conformational change that prevents further access of substrate. Structure-activity studies with more than 300 novel compounds identified zoledronic acid as the lead candidate for clinical development due to its outstanding potency as an inhibitor of osteoclastic bone resorption both in vitro and in vivo, and its excellent in vivo tolerability.

In animal models of breast cancer, prostate cancer, osteosarcoma and haematological malignancies such as multiple myeloma and leukaemia, low doses of zoledronic acid markedly inhibit osteoclastic activity and thus reduce tumour-induced osteolysis and hypercalcaemia. Bone is a rich source of growth factors which are released during bone resorption and can stimulate tumour cell proliferation. By inhibiting osteoclastic activity, zoledronic acid reduces the release of tumour growth factors from bone and thus interrupts this stimulatory cycle. Furthermore, the high local concentration of bisphosphonate at an osteolytic site may exert direct cytostatic and apoptotic effects on the tumour cells in a bone metastasis. Extensive in vitro data show that zoledronic acid is cytostatic and pro-apoptotic against a variety of human tumour cell lines. Interestingly, zoledronic acid enhances the efficacy of some cytotoxic drugs in a synergistic manner, especially when the drugs are administered sequentially rather than concomitantly. Apart from these direct effects on tumour cell proliferation and viability, N-BPs also impair the metastatic behaviour of tumour cells and modulate the host's response to neoplastic disease. In vitro, zoledronic acid potently inhibits tumour cell invasion through extracellular matrix, decreases cell adhesion to both mineralized and non-mineralized matrices, and disrupts soft tissue angiogenesis. Preclinical data from a variety of animal tumour models demonstrate that, in vivo, these effects translate into reduced tumour load, increased tumour cell apoptosis, and in some cases delayed disease progression with a corresponding survival benefit. Although these effects have been predominantly observed in models of bone metastases in nude mice, emerging data indicate that zoledronic acid can also inhibit the growth of primary soft tissue tumours in transgenic animals by a mechanism that apparently involves inhibition of angiogenesis and disrupted macrophage function.

Recently, another intriguing facet has been added to the pharmacological profile of the N-BPs. It has been known for many years that these compounds interact with the immune system to produce an acute phase response in a significant proportion of patients, especially when administered intravenously, but the molecular mechanism remained elusive. It is now known that this effect is caused by inhibition of farnesyl pyrophosphate synthase in monocytes, leading to accumulation of upstream metab-

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

M. Papotti[a], T. Kalebic[b], M. Volante[a], E. Bacillo[a], S. Cappia[a], G.V. Scagliotti[a]. [a]*University of Turin at San Luigi Hospital, Turin, Italy;* [b]*Novartis Pharma, East Hanover, USA.*

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

Roland Goldbrunner, Niklas Thon, Christian Schichor. *Department of Neurosurgery, University of Munich, Germany.*

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