done on the BN-175 and ROS-1 tumor cell lines as well as on the HUVEC, with different drug concentrations.

Results: There was a direct effect of histamine on all cell lines in vitro, although the BN-175 was more sensitive. In vivo there was a better response rate among the histamine alone and the histamine + melphalan ILPs on the BN rats with a 60% overall response (33% complete response). In the Wag/Rij there was a 25% overall response rate although no complete response was seen. We are currently evaluating the increase in drug uptake by tumoral tissue as compared to normal tissue.

Conclusions: The combination of histamine and melphalan shows a dramatic effect on tumoral response in the ILP setting which hasn't been reported yet. The above findings seem promising not only as a cheaper and safer alternative but, maybe, also as a possibility of treatment for the 25% patients who show no response to the standard TNF-alfa + melphalan treatment.

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High serum levels of total MMP-9 and active MMP-13 are associated with rapid progression in patients with metastatic melanoma

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Matrix metalloproteinases (MMPs) are proteolytic enzymes capable of degrading extracellular matrix. MMPs are involved in cancer progression including tumor growth and survival, angiogenesis and metastasis. The inhibition of their activity by synthetic inhibitors has recently shown survival benefit in the treatment of gastric and renal cancers. We assessed the clinical importance of serum MMP-9 and MMP-13 levels on the outcome and therapy response in patients with metastatic melanoma. 48 patients with stage IV melanoma were treated with dacarbazine or polychemotherapy and interferon-alpha. ELISA and gelatin-substrate zymography and Western blot technique with densitometric analysis were used to measure pre-treatment serum levels of latent and active MMPs. Increased total MMP-9 expression levels (e 321 ng/ml) were associated with increased tumour burden (p=0.027), presence of liver metastases (p=0.019) and decreased median disease-free survival (DFS) when compared to lower expression levels. In patients with high MMP-9 levels (n=8) median DFS was 0.7 months vs. 15.3 months in patients with lower MMP-9 levels (n= 40, p=0.05). Most of the MMP-13 found in the patients' sera was in its active (48 kDA) form, when compared to the sera of healthy volunteers (p<0.0001). Interestingly, after beginning of the therapy, patients with high amounts of active MMP-13 (e 421 pixels, n=38) progressed in a markedly shorter time than those with lower levels (median 2.0 vs. 5.9 months, p=0.029), suggesting that their disease was highly active. Our results suggest that total serum MMP-9 levels can be associated with increased tumour burden and survival parameters and the predominance of active form MMP-13 in serum can predict rapid progression. The evaluation of serum MMP-9 and MMP-13 expression might help to select patients to be included in clinical trials investigating MMP inhibitors as new therapeutic agents in metastatic melanoma.

Tumour biology and targeted therapy

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Quantification of angiogenesis as a prognostic marker in 977 human carcinomas: A critical evaluation of the consensus on histopathological methods for estimation of vascular density

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Introduction: Lately, Chalkley counts have been suggested in a consensus report as the primary method for immunohistochemical evaluation of angiogenesis(1), although most studies have used microvessel density (MVD). We present paired Chalkley and MVD estimates in carcinomas of the prostate (n=272), breast (n=455), bladder (n=107) and lung (n=143).

Materials and methods: The clinical data has previously been published. Formalin-fixed, paraffin-embedded tumour sections were immunostained for the endothelial markers von Willebrand Factor (prostate) or CD34 (breast, lung and bladder). Both the *hot spot*-method for MVD estimates and the Chalkley method were applied to all the tumour sections.

Results: In all tumour types MVD and Chalkley estimates were significantly correlated. In prostate carcinomas high MVD strongly indicated poor prognosis (P<0.0001), whereas Chalkley counts were only able to separate the tumours into different prognostic groups when divided in tertiles (P=0.05). On the contrary, in early breast carcinoma high Chalkley scores were associated with poor prognosis (P=0.003), but not MVD scores (P=0.66).

In bladder carcinoma, high estimates of angiogenesis using both methods surprisingly showed good prognosis and were associated with high degrees of inflammation. Neither of the counts revealed prognostic value in nonsmall cell lung carcinomas, where the vascular pattern clearly indicated that 24% of the tumours with this cancer were non-angiogenic.

Conclusions: Our results support that estimates of angiogenesis are important to prognostic outcome, however, the prognostic power of these estimates is not consistently associated with one of the methods investigated. We highlight methodologically problems with both the MVD and the Chalkley methods, and these problems should be properly addressed before any attempt is made to select either of the methods. Since angiogenic processes in lung and bladder cancer may be different from those occurring in prostate cancer, we suggest that future analyses also focus on measuring angiogenic factors to obtain more information on the biology of angiogenesis.

Reference

[1] Vermeulen PB, Gasparini G, Fox SB, Colpaert C, Marson LP, Gion M et al. Second international consensus on the methodology and criteria of evaluation of angiogenesis quantification in solid human tumours. *Eur.J.Cancer* 2002;38:1564-79.

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Quantitative analysis of p53-targeted gene expression and visualization of p53 transcriptional activity following intratumoral administration of adenoviral p53 in vivo

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To analyze the mechanism of the antitumor effect of an adenoviral vector expressing the *p53* tumor suppressor (Ad-*p53*) *in vivo*, we quantitatively assessed p53-targeted gene expression and visualized transcriptional activity of p53 in tumors in nude mice treated with Ad-*p53*. Human lung cancer (H1299) xenografts established in nude mice were treated by intratumoral administration of Ad-*p53*. The levels of expression of exogenous *p53* and p53-targeted genes *p21*, *MDM2*, *Noxa*, and *p53AIP1* were quantified by real-time reverse-transcriptase polymerase chain reaction (RT-PCR) and induction of apoptosis was observed histochemically on days 1, 2, 3, 7 and 14 after treatment. Expression of mRNAs of exogenous *p53* and p53-targeted genes (except *p53AIP1*) was at its maximum 1 day after Ad-*p53* treatment, then decreased rapidly; apoptosis was evident *in situ* 2-3 days after treatment. We developed a noninvasive and simple method for monitoring the transcriptional activity of exogenous p53 following intratumoral administration of Ad-*p53* in nude mice. We established H1299 cells that express the green fluorescent protein (GFP) reporter gene under the control of p53-responsive *p21* promoter (i.e., the p53R-*GFP* reporter system). Xenografts of these cells in nude mice were treated by intratumoral administration of Ad-*p53*, and the transcriptional activity of exogenous p53 could be visualized as intratumoral GFP expression in real time by 3-CCD camera. Expression of GFP was maximal 3 days after treatment, and it decreased remarkably by 7 days after treatment. We demonstrated that Ad-*p53* treatment rapidly induced p53-targeted genes and apoptosis in tumors. We also succeeded in visualizing p53 transcriptional activity *in vivo*. Quantitative analysis of p53-targeted gene expression by real-time quantitative RT-PCR and visualization of p53 transcriptional activity in fresh xenografts by using the p53R-*GFP* reporter system may be useful in assessing the mechanisms of the antitumor effects of Ad-*p53* and novel therapeutic approaches.