696 ORAL

Management of local recurrences of soft tissue sarcomas in an irradiated field after prior surgery and radiotherapy: the role of TNF-based isolated limb perfusions to achieve limb salvage.

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Background: R ecurrent extremity soft tissue sarcoma (STS) in a previously operated and irradiated area can usually only be managed by amputation. TNF-based isolated limb perfusion (ILP) is an established alternative to achieve limb salvage.

Methods: Prospective database of TNF-based ILPs at the Daniel den Hoed Cancer Center in Rotterdam. Out of 326 TNF-based ILPs between1991-2002, 27 ILPs were done in 24 patients with recurrent STS in a highly irradiated field (50-70 Gy) after prior surgery and radiotherapy. Thirteen patients (54%) had multiple tumors (2->20). All patients were candidates for amputation. This represents a unique experience with a very rare subgroup of patients that present with a desperate clinical problem, for which only amputation can be considered normally.

Results: After 27 ILPs we observed: 6 complete responses (22%), 14 partial responses (52%) and in 7 patients no change (26%). The Mean duration of response was 17.6 months (2 - > 56, at a median follow up of 30+ months. With multiple tumors in 13 patients the local recurrence rate was 38% after ILP, also 38% of the patients developed systemic metastases and died. Limb salvage was achieved in 16 of the 24 patients (67%), but of course not all patients were tumor-free in the extremity at time of death. Regional toxicity was limited and systemic toxicity minimal to moderate, with no toxic deaths.

Conclusion: Amputations in patients with recurrent extremity STS in the irradiated field after prior surgery can be avoided in the majority of patients by TNF-based ILP.

697 ORAL

Assessment of the tolerability, safety and efficacy of increasing doses of peginterferon alfa-2a (40KD) in a randomised study in patients with metastatic malignant melanoma

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Background: Metastatic melanoma, a tumour of the pigment-producing cells in the skin, has a poor prognosis. The median survival of patients with systemic metastases is 6-12 months. Overall response rates to interferon alfa-2a and other agents is 10-15%, suggesting the need for new treatments. The sustained absorption and prolonged half-life of peginterferon (PEG-IFN) alfa-2a (40KD) (PEGASYS) may provide benefits over conventional interferon-alfa in terms of convenience and toxicity.

Materials and methods: This open-label, Phase II trial was conducted in patients with confirmed metastatic malignant melanoma (stage IV AJCC) to evaluate the tolerability, safety and efficacy of 3 doses of subcutaneous PEG-IFN alfa-2a (40KD). Patients were randomised to receive PEG-IFN alfa-2a (40KD), 180 μg (n=48), 360 μg (n=53) or 450 μg (n=50), once weekly for an intended period of 24 weeks or more. The median duration of treatment was 8.1 weeks (range 0.1-72.4 weeks; 20% of patients were treated for >24 weeks).

Results: Patient demographics were similar between groups. More patients required a dose adjustment for safety reasons (adverse events or laboratory abnormalities) in the 360 μg (51%, n=27) and 450 μg groups (41%, n=20) than in the 180 μg group (23%, n=11). Study withdrawal due to an adverse event during treatment was low, but more frequent in the 360 μg group (17%) *vs* the 180 μg (4%) and 450 μg groups (10%). Tumour response data are shown (table). The most frequently reported adverse events were fatigue, pyrexia, rigors, nausea, anorexia, myalgia, headache and dizziness. No differences were measured between groups for the time to achieve a major response or disease progression, or the duration of a complete response. The median patient survival is given in the table.

Dose group	Major response (CR+ PR)	Complete response (CR)	Partial response (PR)	Stable disease (SD)	Progressive disease (PD)	Median days alive (95% CI)
180 μg	6.3%	2.1%, n=1	4.2%, n=2	23%, n=11	65%, n=31	203 (153-330)
360 μg	7.6%	1.9%, n=1	5.7%, n=3	19%, n=10	61%, n=32	295 (189-401)
450 μg	12%	6.0%, n=3	6.0%, n=3	20%, n=10	58%, n=29	237 (198-348)

Conclusions: Overall, PEG-IFN alfa-2a (40KD) 180 μg, 360 μg, and 450 μg, appear to demonstrate similar efficacy to interferon. This study shows that the 180 μg dose is better tolerated and seems to have an immunomodulatory effect in patients with metastatic malignant melanoma. The prolonged serum half-life of the pegylated interferon allows a more convenient once-weekly dosing regimen, thereby improving patient quality of life.

698 ORAL

Evaluation of a clinically applicable post-surgical classification system for primary retroperitoneal soft tissue sarcoma

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Purpose: A prognostic tool enabling comparison of outcomes for patients with primary retroperitoneal soft tissue sarcoma (STS) would be useful for designing clinical trials, comparing results from different institutions and evaluating effects of treatment modalities. In the present study a simple post-surgical classification system based on well documented predictors of survival for retroperitoneal sarcoma is evaluated.

Patients and methods: Based on malignancy grade (low versus high), completeness of resection (complete versus incomplete), and distant metastasis (no metastasis versus metastasis), four classes were defined: I, low-grade/complete resection/no metastasis; II, high-grade/complete resection/ no metastasis; III, any-grade/ incomplete resection/ no metastasis, IV, any-grade/any resection/distant metastasis. The prognostic value of this classification system was analyzed in a population based multi-center group(MCG) of patients with primary RSTS (n=124), and in a cohort of patients treated in a single-(tertiary referral) center (SCG; n=107). Median follow-up was more than 5 years in both groups.

Results: Overall 5-year survival rates were 55% in the SCG, and 43% in the MCG (P=0.015). class III (incomplete resection) was more frequent in the MCG than in the SCG (33% vs 16%; P=0.02). In the SCG, stage-specific 5-year survival rates were 89%, 40%, 26%, and 17% for class I to IV respectively(P

699 ORAL

Synergistic antitumor activity of histamine in combination with chemotherapy in the regional treatment of soft tissue sarcomas

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Background: Soft tissue sarcomas are tumors of mesenchymal origin accounting for about 1% of all adult malignant tumors in the USA. Circa 60% of them affect the extremities and are often large at first diagnosis. Isolated limb perfusion (ILP) with TNF+melphalan is now established as an excellent method to achieve limb salvage in the management of irresectable extremity sarcomas with a response rate of 76% and a limb salvage index of 71%. We used the experimental ILP model in rats in order to evaluate the potential effect of histamine. This inflammatory mediator is formed and stored mainly in the granules of mast cells and basophils, but also in cells of the epidermis, gastric mucosa, neurons within CNS and cells in regenerating or rapidly growing tissues. Histamine's classical effect on fine vessels is the formation of edema by an increase in the flow of lymph and its protein content to the extracellular space and also the formation of gaps between endothelial cells increasing transcapillary vesicular transport.

Methods: Twenty BN and 18 Wag/Rij rats had the BN-175 soft tissue sarcoma and the ROS-1 osteosarcoma, respectively, inserted on the right hind limb. After the tumor reached a volume between 12 and 15mm3 they were submitted to one of the following ILPs: SHAM (n=5 and n=4), melphalan (n=5 and n=6), histamine (n=6 and n=4) or histamine + melphalan (n=6 and n=4). In vitro bioassays of histamine with and without melphalan were

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.

700 ORAL

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

701 ORAL

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

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702 ORAL

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.