Melanoma

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Double isolated limb infusion with cytotoxic agents for recurrent and metastatic limb melanoma

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Metastatic melanoma confined to a limb can be treated effectively using an isolated limb perfusion technique (ILP). A simplified alternative method called 'isolated limb infusion' (ILI) has been developed at the Sydney Melanoma Unit (Australia). This method is essentially a very low flow ILP performed via percutaneously introduced catheters without oxygenation of the limb. Previously we have demonstrated that with ILI similar response rates can been achieved as with ILP using melphalan and actinomycin-D as the cytotoxic drugs. The present study was undertaken to determine if electively performing two ILI procedures increases the frequency and/or duration of responses. Short and long-term results were compared with those for patients who underwent a single ILI in the same period of time. Moreover, the value of a second ILI when progression occurred after a first ILI was assessed.

Between 1992 and 1998, a total of 47 patients underwent a planned double ILI and 14 were treated with a second ILI after a good initial response after ILI. After double ILI 76% of patients experienced Wieberdink Grade III or IV toxicity in the treated limb compared to 52% after a single ILI (p=0.02). Overall response (OR) after the planned double ILI was 88% (complete response (CR) 41%, partial response (PR) 47%, stable disease (SD) 12%, progressive disease (PD) 0%). A CR was demonstrated in 70% of patients who were treated with an interval of 3 weeks or less between the two ILIs, however, this was not statistically higher than in patients with longer infusion intervals (p=0.08). The median duration of response was 18 months (6-60), the median patient survival was 17 months. Response rates after double ILI were similar to those in 81 patients treated with a single ILI (CR 41%, PR 41%, SD 12%, PD 5%). Response duration and patient survival were not significantly different for the two groups of patients. Patients who underwent a second ILI because of progression following their first ILI (n=14) had an OR of 77% (CR 8%, PR69%, SD 23%), with a 5 months (4-11) duration of response.

Since elective double ILI does not increase efficacy but increases toxicity, single ILI is the more appropriate treatment for melanoma confined to the limb. However, a second ILI can be of value if disease recurs or progresses following a previous ILI.

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Safety profile of histamine dihydrochloride administered with interleukin-2 in patients with advanced metastatic malignant melanoma

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Histamine Dihydrochloride (HDC) is being investigated as an adjuvant to interleukin-2 (IL-2) therapy in metastatic melanoma. Safety data were collected for 391 patients (pts) in two multicenter studies of HDC (Ceplene) administered with IL-2 in pts with stage IV malignant melanoma (IL-2 + HDC: n=239; IL-2 only: n=152).

Methods: Pts received IL-2 (9 MIU/m2, bid, sc, days 1-2, weeks 1,3; and 2 MIU/m2, bid, sc, days 1-5, weeks 2,4) with or without histamine (1.0 mg, bid, sc, days 1-5, weeks 1-4) for 4 weeks of a 6-week cycle. All therapy was administered in an outpatient at home setting. Safety and toxicity were assessed according to NCI Common Toxicity Criteria in all pts who received at least one dose of study drug.

Results: Common, anticipated toxicities, including fever, chills, asthenia, nausea, vomiting, anorexia, pain, diarrhea, cough, dyspnea, rash, and injection site pain, were reported with similar frequency and intensity (grade 1-4) among the two treatment groups. The following toxicities were observed with greater frequency in the IL-2/HDC group: vasodilation (94% vs. 36% of pts experiencing at least one episode), headache (59% vs. 35%), hypotension (51% vs. 20%), injection site inflammation (49% vs. 22%), injection site reaction (48% vs. 23%), rhinitis (38% vs. 25%), and dizziness (36% vs. 23%). In addition, clinically significant grade 3 and 4 toxicities occurred with similar frequency among treatment groups, with the lone exception being grade 3 headache, observed in 8% of patients receiving IL-2/HDC vs. 2% in the IL-2 group. Dose reduction (14% vs 14%), interruption (28% vs. 29%), and discontinuation (11% vs. 11%) of study drug administration were comparable among treatment groups. The rate of on-study death, which includes deaths due to progressive disease and deaths occurring within 28 days of final study drug administration, was 11% (27/239) in the IL-2/HDC group, compared to 16% (25/152) in the IL-2-alone group.

Conclusions: This review of IL-2/HDC safety and toxicity data suggests that HDC may be safely administered in conjunction with a regimen of low-dose sc IL-2, and adds low clinically significant grade 3 or 4 toxicity.

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A novel mutation of the mismatch repair gene hMLH1 in a human primary skin melanoma

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Purpose: Cells with a defective mismatch repair system (MRS) display high rates of spontaneous mutations, microsatellite instability and resistance to O6-guanine methylating agents and some other drugs. We identified a human melanoma cell line (PR-Mel) expressing almost undetectable levels of the DNA repair enzyme O6-alkylguanine-DNA alkyltransferase and still highly resistant to the cytotoxic activity of the methylating agent temozolomide. We therefore investigated whether PR-Mel tolerance to temozolomide was associated with a defective MRS.

Methods and Results: PR-Mel cell line was analyzed for mismatch repair activity, expression of MRS proteins and microsatellite instability at 12 different loci. The PR-Mel cell line was completely devoided of mismatch repair activity, did not express the hMLH1 and PMS2 proteins, and possessed high levels of microsatellite instability. We evaluated whether in PR-Mel cell line was present an aberrant hMLH1 promoter methylation or whether this line harbored mutations in hMLH1. No promoter methylation was found. Sequence analysis of all exons of hMLH1 revealed the presence of a G to A transition at position ñ1 of the acceptor splice site of intron 15. The mutation, named 1732-1G->A, alters the correct splicing of hMLH1 pre-mRNA leading to the in-frame skipping of exon 16, as attested by RT-PCR analysis. The somatic mutation 1732-1G->A was also detected in genomic DNA from both the primary skin melanoma and the cutaneous metastasis from which PR-Mel cell line had been established. Immunohistochemistry demonstrated no expression of hMLH1 and PMS2 in the tumor specimens. Both the cell line and biopsies appeared homozygous for the mutation, suggesting that the normal allele was lost. Indeed, cytogenetic analysis of PR-Mel cells revealed a 3p deletion possibly including the hMLH1 gene.

Conclusions: A cluster of hMLH1 mutations has been described in the region encompassing exons 15 and 16. However, mutation 1732-1G->A has not been previously described. Our data therefore expand the repertoire of hMLH1 mutations. To our knowledge, this is the first study in which a mutated MRS gene has been identified in a human melanoma.

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Treatment of chemoresistant metastatic malignant melanoma (MMM) with cationic colloidal BCL-2 antisense ODN (SEVINA-22) and vinorelbine-tartrate induces apoptosis via caspase-3 (CPP32) pathway

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Purpose: Metastatic malignant melanoma (MMM) are highly resistant to conventional cancer treatment including radiation and chemotherapy due to overexpression of bcl-2 oncogene which occurs in over 90% of all melanoma cases and acts as a negative regulator of apoptosis. We aim to induce apoptosis in chemoresistant MMM by combined chemogene treatment consisting of bcl-2 antisense ODN and vinorelbine.

Methods: We obtained melanoma cells by FNA of metastatic lesions in a patient with MMM disease. IHC analysis exhibited bcl-2 overexpression. RT-PCR amplification for bcl-2 mRNA was performed and positive results were obtained. An 18 mer phosphorothioated antisense oligodeoxynucleotide (ODN) containing unmethylated CG-dinucleotides (CpG motifs) against the bcl-2 messenger RNA was entrapped inside pegylated liposomes composed of DOTAP, DOGS, DDAP and DOPE. This colloidal bcl-2 antisense

ODN complex was code named as SEVINA-22. MMM cells were treated with SEVINA-22 and vinorelbine-tartrate.

Results: Post-treatment analysis by IHC and RT-PCR exhibited no expression of bcl-2 and negative results after amplification for bcl-2 mRNA. Thus, the short DNA-like strand carried by the cationic liposomes targeted efficiently the bcl-2 messenger, preventing it from being translated into protein. Vinorelbine induced apoptosis (VIA) detected by annexin-V/PI staining was mediated by activation of caspase-3 (CPP32) pathway, blockage at G2/M phase and interupting mitochondria transmembrane potential releasing cytochrome (cyto)-c according to flow cytometric analysis. TEM has exhibited morhological signs of D2 apoptotic stage forming melanoma apoptotic bodies (MABs) which were phagocytosed by adjacent MMM cells leading to a bystander killing effect. MTT and BrdU analysis of treated MMM cells exhibited greatly reduced metabolic activity and DNA synthesis, respectively.

Conclusion: Thus, SEVINA-22 by sequence hybridization in bcl-2 mRNA has inhibited bcl-2 expression allowing vinorelbine-induced apoptosis (VIA) in chemoresistant MMM cells. Concluding, this antisense strategy in combination with the antimitotic drug can eradicate chemoresistant MMM cells.

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Genetic analysis of sporadic and familial malignant melanoma (MM): assessment of the role of 9p21 region and p16/CDKN2A gene in MM tumorigenesis and its clinicopathological correlation

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Background: Previous studies indicated the 9p21 as the chromosomal region involved in MM pathogenesis. In addition to the CDKN genes (p16/CDKN2A and p15/CDKN2B, frequently inactivated in familial MM), presence within this region of other melanoma susceptibility gene(s) has been suggested. To assess the role of 9p21 in MM, genetic alterations in primary tumors as well as in their synchronous or asynchronous metastases were evaluated by PCR-based analysis using polymorphic markers.

Patients and Methods: Genomic DNA was extracted from archival paraffin-embedded samples after microdissection separating tumor cells from normal adjacent tissues by light microscopy. Loss of heterozygosity (LOH) was defined by the absence of one allele in the tumor sample after comparison to the heterozygous normal tissue genotype. Microsatellite instability (MSI) was defined by the presence of additional bands in the PCR-amplified product from tumor DNA compared with normal DNA.

Results: LOH and MSI were found in 27 (41%) and 11 (17%), respectively, out of 66 primary tumors. In corresponding 58 metastases, MSI was found at higher rate (22; 38%), whereas a quite identical pattern of LOH (27; 47%) was observed. Although CDKN locus was mostly affected by LOH, an additional region of common allelic deletion at D9S171 was identified. This region was also confirmed by PCR analysis on primary tumor cell lines obtained from MM patients (D9S171 was found homozygously deleted). In few cases (when peripheral blood sample was available) with LOH at the CDKN locus, no germline mutation was detected by direct sequencing of the p16/CDKN2A exons, suggesting alternative mechanisms which inactivate these genes on the retained alleles at somatic level.

Conclusion: Although no correlation between 9p21 rearrangements and clinicopathological parameters was observed in our series, further mutational screenings in candidate genes among sporadic and familial MM patients are being performed to identify genetic alterations with prognostic significance.

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Conservation treatment of the eye: conformal proton re-irradiation for recurrent uveal melanoma

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Purpose: Evaluate outcome of second course of proton irradiation (PI) in patients with recurrent uveal melanoma.

Patients and methods: 31 patients (10 male and 21 female) had a second course of PI. Mean patient age at the time of the second treatment was 66.1 years (range 45-84). Mean interval between the first and the second PI course was 50.2 months (range 8-165). One patient received a third course of PI, 37 months after the second treatment.

Largest tumor diameter and thickness were 13.2 mm (range 6-21) and 5.2 mm (range 1.1-13.9) respectively, at presentation, and 14.6 mm (range 4.5-24.1) and 5.5 mm (range (2-8.2), respectively, at second treatment. None had distant metastasis.

Doses for the first course were 70 CGE (28 pts) and 50 CGE (3 pts), 70 CGE (30 pts) and 48 CGE (1 pt) for the second, and 70 CGE (1pt) for the third course (CGE= proton Gy x 1.1). 30 patients received 5 fractions and one (48 CGY) had 4 fractions. Approximate percent overlap between the first and the second course ranged between 40 and 60% in 12 patients, between 70 and 90% in 5 patients and was 100% in 15 patients.

Visual acuity was 20/200 or better in 30 patients initially and in 22 at second treatment.

Mean follow up time after the second treatment was 50 months (range 6-164).

Results: At the time of the last follow up, 20 patients survived without recurrence or metastasis, 3 were surviving with metastasis, 5 had died of melanoma metastasis, and 3 of other causes. The five-year metastasis-free survival rate was 73% (95% CI: 45-89%). The cumulative rate of local recurrence at 5 years was 31% (95% CI: 11.4 ± 68.1%).

In total, nine eyes (29%) were enucleated, due to either local recurrence (n=5) or intractable pain (n=4). The 5-year eye retention rate was 55% (95% CI: 25.2 ± 77.4%).

Of the remaining 22 patients 6 (27%) had useful vision, and 12 (54%) had radiation induced cataract.

Conclusion: A second course of proton irradiation for recurrent uveal melanoma to total doses between 118 and 140 CGE was associated with a relatively low enucleation rate due to serious complications, and a good probability of local control.

Although most patients lost vision, the majority was able to retain the re-irradiated eye.

Further evaluations are needed to compare the influence on metastasis free survival of additional proton irradiation versus enucleation after local recurrence.

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Melanoma and pregnancy: a poor prognosis?

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Purpose: Cutaneous Melanoma are aggressive tumours with an unpredictable biological behaviour. Tumour site, tumour thickness according to Breslow, tumour ulceration and vascular invasion are the most important prognostic factors. Over the years it has been suggested that women who develop a melanoma during or shortly after a pregnancy have also a worse prognosis due to a more aggressive behaviour of the melanoma. We therefore embarked on a retrospective analysis of the pregnant women with a melanoma.

Materials and Methods: During the period 1965-2001, 67 pregnant women (P) were diagnosed and treated for a melanoma. These patients were subsequently compared with a control group (C) of 544 female melanoma patients in their reproductive phase of life. The melanoma were staged according to the MD Anderson staging system and the clinical and pathological data was retrieved from the Groningen melanoma database. The 10-year disease free interval (DFI) and 10-year survival were calculated using the Kaplan-Meier method.

Results: There were in total 54 P stage I pts. compared 457 C stage I pts.; 3 P stage II pts. with 30 C stage II pts.; 9 P stage III pts. with 82 C stage III pts.; 5 P stage IV with 10 C stage IV pts.

De median age for the P group was 30.0 (range 18.5-46.5) years and 35.9 (range 16.6-45.0) years for the C group. The median follow up time for both groups combined was 95 (range 1-398) months. There was no statistical difference between the two groups for tumour thickness, tumour localisation, tumour ulceration and vascular invasion. The 10-year DFI for the 2 groups calculated resulted in no statistical difference (st.I 79% P vs. 80% C, st.II and III 44% P vs. 49% C). This was also the case for the 10 year survival (st.I 92% P vs.92% C, st.II and III 72% P vs. 66% C).

Conclusion: Pregnancy is not a risk factor for the prognosis of melanoma. The prognosis is still dependent on the tumour localisation, thickness, ulceration and vascular invasion.