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.

288 POSTER

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.

289 POSTER

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.

290 POSTER

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.