Mel 09

G_{D3} GANGLIOSIDE: A MARKER FOR THE DIAGNOSIS AND TREATMENT OF NEUROECTODERMAL TUMORS?

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Monoclonal antibody (mAb) R-24, recognizing ganglioside G_{D3} on neuroectodermal tumors, shows several functional properties in vitro: inhibition of cell growth, complement fixation and mediation of antibody dependent cellular cytotoxicity (ADCC). The tissue specificity of this mAb R-24 was studied on 190 cryopreserved, unfixed human tissue sections by indirect immunoperoxidase staining. The specificity for neuroectodermal tumors was striking. Ganglioside G_{D3} was detected in all 21 tissue sections of 21 patients with primary melanoma and in all 37 probes of 24 patients with metastatic malignant melanoma. The majority of tumor cells in the samples of primary malignant melanoma expressed G_{D3}: however, G_{D3} expression was more heterogeneous in samples of metastatic lesions even in different metastases of the same patient. Of 18 nevi, 16 reacted with monoclonal antibody R-24, while melanocytes in the basal layer of normal skin stained only weakly and irregularly. None of the 32 normal and 12 fetal human tissue types were R-24 positive, but a strong cytoplasmic staining was observed with single cells in the dermis and in the interstitial tissue of the gastrointestinal tract, in the interlobular septa of the thymus, and in other distinct locations. Only two malignant carcinoid tumors of 38 nonmelanomatous tumors tested reacted with monoclonal antibody R-24. The presented findings are particularly relevant in view of the clinical application of this mAb in vivo. Local inflammatory response at the tumor site and tumor remissions have been observed after application of purified mAb R-24 in a percentage of patients. Side effects (skin rash) have been minimal with this specific antibody.
New monoclonal ganglioside antibodies have been generated to cover the complete antigenic phenotype of neuroectodermal tumors.

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Mel 10

COMPARISON OF THE IN SHORT-TERM METABOLIC ASSAY AND IN VIVO TREATMENT OF HUMAN MELANOMA XENOGRAFTS

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Histology and stage are major prognostic factors for the treatment of neoplastic disease but tumors with similar histology may respond heterogeneously to antineoplastic drug treatment. For the short-term metabolic assay extensive claims have been made as to its general applicability as a predictive test, although only two drugs are used to indicate sensitivity or resistance. This contrasts sharply with findings of other investigators who describe unique chemosensitivity patterns in xenografted human tumors (1). We have exposed 3 human melanoma xenografts to alkylating agents and adriamycin and compared tumor growth curves to results from short-term assays performed on these xenografts. 'Str' is about 100fold more sensitive to dacarbazine than 'GrII' and may be cured by intermediate doses of methyl-CCNU. Adriamycin is only very weakly effective against this tumor, and an activated form of cyclophosphamide, mafosfamide (ASTA Z 7557), induces some growth delay. Methyl-CCNU, mafosfamide, and adriamycin were totally ineffective against melanoma 'JaI', although the latter line is somewhat sensitive to dacarbazine.
Taking a 50% inhibition of ^{3}H-uridine (or ^{3}H-thymidine) incorporation as a cutoff-point, tumor line 'Str' was correctly classified as sensitive to methyl-CCNU and mafosfamide but falsely as sensitive to adriamycin. All three drugs were falsely classified as effective for completely resistant melanoma 'GrII', and for line 'JaI' only resistance to methyl-CCNU was indicated correctly by the short-term assay. Therefore, the limitation of predictive testing to one or two key antineoplastic drugs is not warranted by the heterogeneity present even among human tumors of similar histiogenetic origin.

(1) Osieka, R: Primary and acquired resistance to antineoplastic chemotherapy. Cancer 54: 1168-1174 (1984)

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Mel 11

T-LYMPHOCYTOTOXICITY IN MALIGNANT MELANOMA: INDUCTION WITH AUTOLOGOUS MUTAGENIZED TUMOR CELL CLONES

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Mutagen treatment of spontaneous non-immunogenic mouse tumors induces variants that elicit a strong rejection response in syngeneic mice. The rejection response is due to so-called tum$^-$ antigens recognized by cytolytic T-lymphocytes (CTL). After rejection of tum$^-$ tumor cell clones, mice show a certain degree of resistance against a challenge with the original non-mutagenized tumor cells (tum$^+$). Our study was to investigate if this phenomenon of immune protection would have relevance in human cancer immunotherapy. Melanoma cells from a 38 year old patient with metastatic melanoma were adapted to tissue culture (MZ-MEL-2), cloned and repeatedly mutagenized in vitro with mutagenic compounds. After several intracutaneous immunizations with lethally irradiated mutagenized autologous tumor cell clones peripheral blood mononuclear cells (PBL) were cultured in vitro with these melanoma clones, expanded in IL-2 containing culture medium and tested for cytolytic activity and specificity in vitro. Strong CTL activity directed against the autologous melanoma cells, but not Epstein-Barr-virus transformed autologous B-lymphocyte cultures, K562 cells or allogeneic melanoma targets was seen. Mutagenized as well as non-mutagenized autologous melanoma target cells were lysed. The specificity of non-cloned CTL was confirmed in competitive inhibition assays. We are presently investigating, if CTL bulk cultures or CTL clones after coculture with different mutagenized autologous tumor cell clones will preferentially lyse a single tumor clone, and not the non-mutagenized original tumor cells (tum$^+$). This would be the evidence of a mutagen induced expression of a new antigen (tum$^-$). The clinical course of this melanoma patient evolved remarkably well under continued vaccination with repeatedly mutagenized autologous melanoma cell clones. An abdominal tumor mass gradually disappeared. The patient remains in complete remission for over twelve months. A correlation between the unexpectedly favorable clinical course and the occurrence of an anti-tumor CTL response is suggested.

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Mel 12

A RANDOMIZED PHASE-III-STUDY ON DTIC, LEVAMISOLE, and PLACEBO AS ADJUVANT TREATMENT OF HIGH RISK STAGE I PRIMARY MELANOMA

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This study was designed to assess the value of chemotherapy vs. immunotherapy in high risk melanoma after surgical removal of a primary malignant melanoma of the skin. Eligibility criteria included the following conditions: previously untreated, histologically confirmed, primary cutaneous malignant melanoma of Clark stage III, IV, V with a Breslow's thickness equal or exceeding 1,5 mm, no evidence for lymphnode metastases. Only melanoma of the extremities were submitted to elective lymphnode dissection. Randomization for 3 arms was done between DTIC and immunotherapy plus placebo. DTIC (250mg/m^2 b.s./dayx5, q4w) was given for a total of 6 cycles. Levamisole or placebo was taken orally on 2 consecutive days every week for 2 years (150-250 mg tablets per day according to weight). Results: 235 patients were entered and 94 considered as ineligible. Subgroup analysis reveals that the 96 limb melanoma patients did better than the 172 head, neck, and trunk patients: 5 years DFI 70% vs. 45% and 5 years S 75% vs. 50% resp. This highly significant difference may be due in part to the histological selection of node-negative patients for limb melanoma. 107 out of 276 eligible patients reported a progression, which was local in 19, regional in 47 and distant in 41 patients.
Toxicity has been acceptable but higher in the DTIC arm where more protocol violations occured.
This report indicates that neither DTIC for 6 months not Levamisole for 2 years had any effect on disease free interval (DFS) or survival (S) of patients with high risk primary melanoma.

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