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RADIOIMMUNODETECTION AND RADIOIMMUNOTHERAPY OF MALIGNANT MELANOMA

A review

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Radioimmunodetection utilizing monoclonal antibodies to various melanoma-associated surface antigens has been studied by several investigators during the past ten years. In the early trials, antibodies were labeled with ^{131}I or ^{111}In, but now ^{99m}Tc is almost exclusively used because of its more favorable energy for gamma camera imaging. Excellent specificity has been achieved in most studies, whereas sensitivity has been less good. In a recent European multicenter study on 493 patients sensitivity was 79% and specificity 96%. In this largest study on melanoma so far performed many previously unknown metastatic deposits were identified indicating that radioimmunodetection has a role in the management of metastatic disease. The clinical utility of immunoscintigraphy in localization of regional lymph node metastases has been documented in several investigations in recent years, indicating that this method can be used in the preoperative evaluation of patients. Radioimmunodetection has also been successfully used in the differential diagnosis of ocular lesions. However, conclusive evidence of improved patient outcome resulting from the earlier detection of melanoma lesions by immunoscintigraphy is still lacking. Anti-melanoma antibodies labeled with alpha- and beta-emitting isotopes are potential therapeutic agents, but so far there is little clinical experience with radioimmunotherapy of metastatic melanoma.

Various radiopharmaceuticals, including ^{125}I-labeled chloroquine analog (1), ^{67}Ga-citrate (2), ^{111}In-bleomycin (3) and ^{131}I-meta-iodobenzylguanidine (4), have been tried as imaging agents for cutaneous melanoma, but with limited success due to low diagnostic sensitivity or specificity.

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Holman et al. (5, 6) reported that mouse melanoma cells accumulate radioiodinated iodoamphetamine in vitro and that this compound is taken up in the area of ocular melanin production in various animals including primates. This radiopharmaceutical was therefore considered by us a potential agent for imaging of melanoma and was administered to three patients with cutaneous melanoma metastases (7). In two of the patients, metastases accumulated this tracer but a stronger uptake of ^{99m}Tc-labeled monoclonal antibody (MAB) was found when comparing target-to-nontarget uptake ratios. These results made us proceed with radioimmunodetection (RID) of melanoma, whereas no further trials were conducted with iodoamphetamine. Subsequently only 11 patients with cutaneous melanoma (8), and a singular case of ocular melanoma (9), studied with iodoamphetamine have been reported. From this fact we infer that imaging of malignant melanoma with iodoamphetamine has less clinical utility than RID.

In this article we have reviewed the literature on studies with radiolabeled MABs for the diagnosis and therapy of malignant melanoma, including a European multicenter study, with information also on patients studied by us, and an American multicenter trial.

Radioimmunodetection of cutaneous melanoma

The first clinical investigations with radiolabeled anti-melanoma antibodies were conducted by Larson et al. (10), who were able to visualize 88% of lesions larger than 1.5 cm in six patients with metastases. They employed ^{131}I-labeled monoclonal antibodies directed against a cell surface oncoprotein, p97, identified in human melanomas and certain other neoplasms (11).

In a European multicenter trial, the largest RID study so far published (12), 493 patients were investigated with the murine monoclonal antibody 225.28S recognizing a high molecular weight melanoma-associated antigen (HMW-MAA) expressed in more than 90% of neoplastic melanoma lesions and detectable also in the blood. The antibody, which belongs to the IgG_{2a} class, was originally prepared from human melanoma cell cultures by the Ferrone group (13). This study, conducted with ^{99m}Tc-labeled $F(ab')_2$ fragments, involved 20 nuclear medicine departments in 11 countries. Of a total of 728 lesions, 70%, including 123 previously occult metastases, were visualized by RID. About 70% of the lesions not visualized were less than 2 cm in size.

Similar performance has been observed in an earlier Italian multicenter RID study comprising 258 melanoma patients (Table), a study which demonstrated that more lesions can be detected with ^{99m}Tc than ^{111}In label (14). A significantly higher rate of false negative findings were obtained in stage IV melanoma than in other disease stages, probably because of a high incidence of necrotic or poorly vascularized lesions in advanced disease, and partly also due to lack of expression of the antigen at a late stage. Using the ^{111}In-labeled murine monoclonal antibody ZME-018, directed against a high molecular weight melanoma-associated glycoprotein, Taylor et al. (15) observed 78% sensitivity and 71% specificity when studying 25 melanoma patients, 18 with active disease.

In an American multicenter study (16), 145 patients were imaged with ^{99m}Tc-labeled Fab fragments of an antibody developed against NR-ML-05, a 250 kD glycoprotein-proteoglycan melanoma antigen. The detection rates for deep lesions <1 cm, 1–3 cm and >3 cm in size were 41%, 67% and 82% respectively. The detection rate for all superficial cutaneous lesions, the majority of which were smaller than 5 mm, was only approx. 20%, but for subcutaneous and lymph node lesions combined the detection rate was 77%.

Recently good results were reported by Blend et al. (17), using the same ^{99m}Tc-labeled antibody fragments as in the American multicenter trial, when studying 12 patients, 9 of whom were found to have malignant melanoma when all sites suspected by clinical investigation and RID were examined microscopically.

Specificity has been good in the majority of melanoma RID studies, but there is apparently a need for improved sensitivity. Better sensitivity after pretreatment of melanoma patients with irrelevant antibody not competing for specific antigenic binding sites has been achieved, probably because of the prolonged half-life of the labeled monoclonal antibody fragments, resulting in an enhanced uptake of radioactivity in melanoma lesions (18, 19). Other efforts to improve the diagnostic sensitivity include optimization of antibody and radioactivity doses (20) and the use of a cocktail of two antibodies to different antigenic

Table

Summary of results from studies on radioimmunodetection of malignant cutaneous melanoma. The performance characteristics for lesion detection

Authors/(Reference)	Number of patients	Sensitivity %	Specificity %	Accuracy %
Siccardi et al. (12)	493	79	96	84
Siccardi et al. (14)	254	84	92	86
Feggi et al. (23)	85[a]	83	99	
Feggi et al. (23)	50[b]	62	93	
Eary et al. (18)	27	81	100	
Cerny et al. (22)	25	59		
Pyrhönen et al. (27)	23	68	100	
Murray et al. (20)	21	60		
Lamki et al. (19)	20	79		
Blend et al. (17)	12	91		
Larson et al. (10)	6	80	100	

[a] Follow-up patients
[b] Preoperative patients

epitopes, an approach tested in human melanoma-bearing nude mice without any apparent increase in the specific tracer localization in melanoma lesions as compared with injection of a single antibody (21). Importantly, interferon did not induce or enhance expression of HMW-MAA as judged by immunohistochemistry and did not affect the imaging results in melanoma patients (22).

In a group of 50 preoperative patients studied by Feggi et al. (23) with ^{99m}Tc-F(ab')$_2$-fragments of the 225.28S antibody, sensitivity was 61.5% and specificity 93.3% in assessing regional lymph node involvement, whereas in the follow-up of another group of 85 patients these performance characteristics were markedly higher, 83.3% and 98.8% respectively. In the follow-up group RID visualized all previously documented melanoma lesions, and also disclosed new lesions in three patients.

Wahl et al. (24) evaluated a lymphoscintigraphic approach by administering a cocktail of two ^{131}I-labeled monoclonal antibodies to melanoma antigens at the site of the primary lesion, or its postoperative scar, in 11 patients with stage I or II disease, demonstrating the potential usefulness of the method. Less promising results were obtained in earlier radioimmunolymphoscintigraphy studies utilizing ^{111}In-labeled antibody because of retention of antibody also in unaffected lymph nodes (25, 26).

Own experience. RID was performed on 23 patients with histologically verified metastatic melanoma using ^{99m}Tc-MAB (225.28S) F(ab')$_2$-fragments (27). This study was part of the European multicenter trial (12). RID detected all known metastases in 8 patients, 5 of whom had only one lesion, and was positive in an additional 6 patients. Detectability of metastases in the 14 cases with positive scintigrams were as follows (total number of lesions in parentheses): cutaneous and subcutaneous 92% (n = 13), regional lymph node 79% (n = 14), bone 71% (n = 7), chest 50% (n = 6), and abdominal 20% (n = 5). No false positives were recorded. RID was negative in 9 patients with a total of 20 known lesions. Of 15 lesions less than 2 cm, 7 were detected and of 46 lesions over 2 cm, 27 were localized showing that tumor size was not a major determinant of detectability. Apparently this method is quite sensitive for the detection of metastases in superficial regions and regional lymph nodes, but less sensitive for the detection of deep metastases, possibly partly due to the fact that we were unable to apply single photon emission computed tomography (SPECT) systematically due to limited equipment capacity. Flow cytometric analysis of the metastases revealed that 7 out of 8 (88%) patients with diploid tumors had positive RID, whereas only 7 of 15 (47%) patients with aneuploid tumors were RID positive ($p = 0.056$). This is so far the only report on an association between outcome of RID and flow cytometric DNA-ploidy. One would expect diploid tumors to be more prone to express the high molecular weight antigen used for antibody production as this antigen is known to occur also in benign naevi (13). Immunostaining for the antigen, performed on samples from 19 patients, was positive in all cases studied except for one RID negative case, but there was no correlation between the intensity of staining and lesion detectability. However, it is still possible that the different outcome of RID in aneuploid and diploid tumors could have been caused by less antigen being expressed in aneuploid tumors, but since a quantitative melanoma antigen assay was not available this assumption could not be adequately tested. Nevertheless, these data indicate that the diagnostic usefulness of anti-melanoma RID may be improved by selecting patients not only by testing for the existence of the appropriate antigen in blood, or in an accessible lesion, but also on the basis of DNA analysis of a tumor sample.

Radioimmunodetection of ocular melanoma

The 225.28S antibody has also been used successfully for differential diagnosis of ocular tumors (28, 29). RID was positive in 41% of 37 patients with ocular melanoma when planar imaging was used, but when SPECT was employed the rate of true positive findings rose to 73% (29). Bomanji et al. (30) reported uptake of antibody in a benign naevus in a patient with RID positive choroidal melanoma in the other eye, whereas the two patients with benign naevi studied by Scheidauer et al. (29) were RID negative showing that RID has good specificity also in ocular melanoma.

Own experience. We studied 4 patients with suspected intraocular melanoma, employing ^{99m}Tc-labeled 225.28S antibody, by performing planar imaging (low energy general purpose and pinhole collimators) and SPECT in all cases. In the three patients with choroidal melanoma, verified histopathologically in two cases, RID was positive (Figs 1 and 2) (data not previously published). In one patient with negative RID the diagnosis is probably melanoma, but histological verification is so far lacking as the patient has been treated only by radiotherapy.

Radioimmunotherapy

Therapeutic amounts of ^{131}I-labeled anti-p97-antibody Fab fragments have been administered by Larson et al. (31) to patients with strong uptake of diagnostic doses of antibody. Objective anti-tumor effects were observed in two of three patients who received 16–32 GBq doses, whereas none of the 7 patients who received smaller doses showed any therapeutic response (32). We are not aware of reports on clinical radioimmunotherapy of melanoma from other research centers. Neutron capture therapy based on transport of boron to melanoma cells using ^{10}B-monoclonal melanoma antibodies is also presently being developed (33).

Concluding remarks

Radioimmunoscintigraphy of malignant melanoma has utility in the presurgical evaluation of patients as lesions not

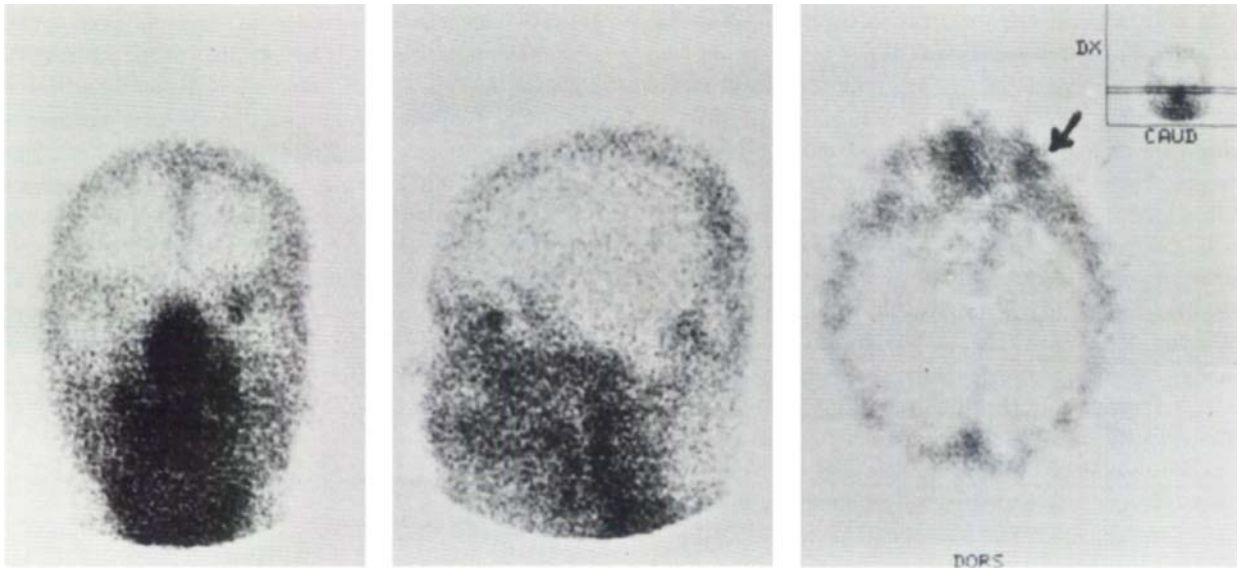

Fig. 1. Localization of choroidal melanoma in left eye of a male patient aged 47 using ^{99m}Tc-labeled monoclonal antibody to a melanoma-associated antigen 4–5 h after intravenous administration of antibody. Gamma camera imaging in anterior (left panel) and left lateral projections (middle panel) and single photon emission computed tomography (SPECT, right panel) were performed with a parallel hole collimator.

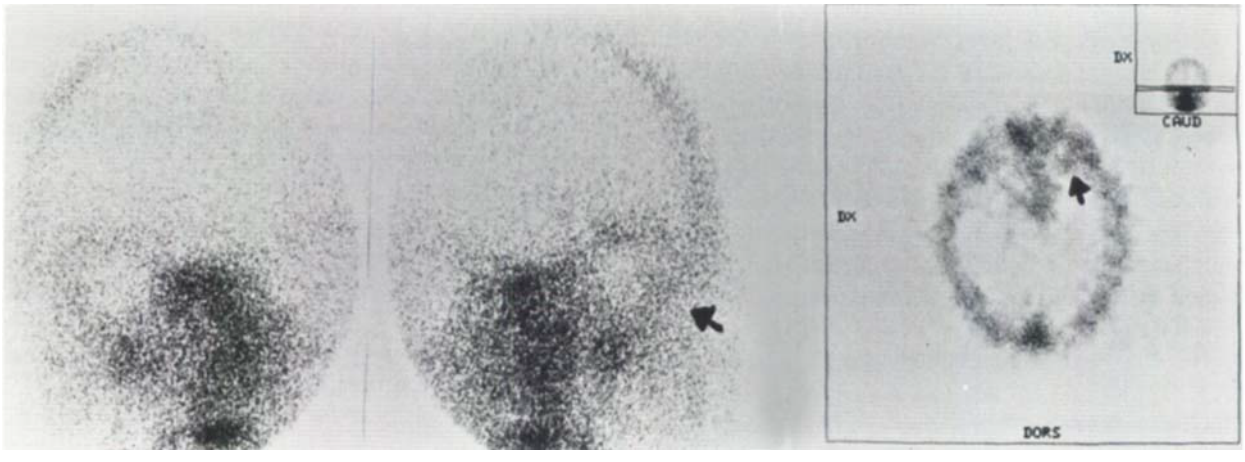

Fig. 2. Uptake of ^{99m}Tc-labeled antibody in choroidal melanoma in left eye of a female patient aged 65 at 4–6 h postinjection. Gamma camera imaging in anterior projections (left and middle panels) was performed with a pinhole collimator and for SPECT (right panel) a parallel hole collimator was used.

recognized by conventional staging procedures can sometimes be detected. The best results have been achieved using ^{99m}Tc-labeled murine monoclonal antibody fragments showing little or no adverse effects even on repeated administration to patients with a human antimurine antibody response. The specificity of melanoma immunoscintigraphy is excellent and better than for most other oncological radioimmunodetection methods, but there is a need for improvement of sensitivity before a more general clinical application of the method can be anticipated. Most of the false negative results are due to small superficial lesions and hepatic or other stage IV metastases, whereas the detection rate is acceptable for subcutaneous and lymph node lesions. The more frequent false negative results in advanced disease are apparently of little practical significance as therapeutic measures have only limited or no effect on outcome, whereas patients with regional lymph node metastases are potentially curable and should therefore benefit from radioimmunodetection.

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