

Autoimmune Toxicities Associated with the Administration of Antitumor Vaccines and Low-Dose Interleukin-2

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Summary: The purpose of this investigation was to evaluate the occurrence of autoimmune toxicities associated with the administration of low-dose IL-2 in conjunction with vaccines for melanoma. Ninety-three patients with stage IIB, III, or IV melanoma were enrolled in three clinical trials and received anti-melanoma vaccines on days 1, 8, 15, 29, 36, and 43. The vaccines comprised peptide-pulsed dendritic cells, autologous tumor cells with GM-CSF in Montanide ISA-51, or synthetic peptides with GM-CSF in Montanide ISA-51. In conjunction with the vaccines, all patients were administered 3×10^6 IU/m²/d IL-2 subcutaneously for 42 days, either days 8 to 49 or 29 to 70. Clinical and laboratory data from these studies were reviewed in aggregate to evaluate the occurrence of autoimmune toxicities. Of 91 evaluable patients, vitiligo was documented in 6 patients (7%). In addition, one patient experienced transient severe insulin-dependent diabetes that resolved after discontinuing IL-2, and another experienced an exacerbation of his pre-existing diabetes; these occurrences are consistent with an autoimmune insulinitis. Four occurrences (4%) of transient minor ocular toxicity were documented, but no autoimmune ocular toxicities or changes in visual acuity were found. Of 55 evaluable patients, 14 experienced thyroid abnormalities (25%). These were attributed to an autoimmune thyroiditis, which was supported by findings of antithyroid antibodies in three of the seven patients evaluated. Overall, autoimmune toxicities

affecting several organ systems were observed in patients receiving melanoma vaccines in conjunction with low-dose IL-2. None of these toxicities caused major long-term effects, though one was acutely life-threatening and others contributed to treatment-related morbidity. Peptide- or cell-based vaccines administered in combination with low-dose IL-2 appear to be capable of breaking tolerance to self-antigens; despite the associated toxicities, these combinations may still be useful to administer as an immunotherapy for cancer. However, careful monitoring for autoimmune toxicities should be incorporated in future clinical studies incorporating low-dose IL-2.

Key Words: toxicity, IL-2, immunotherapy, melanoma, tumor vaccines (*J Immunother* 2005;28:412–419)

High-dose IL-2 aids the generation of antitumor responses *in vivo*, can induce clinical tumor regression, and is approved by the FDA as a treatment of metastatic melanoma. This therapy has an overall tumor response rate of 15% to 20%, with 5% being durable complete responses. However, the antineoplastic potential of high-dose IL-2 is limited by its frequent and severe toxicities.^{1–7}

When administered in high doses, IL-2 binds high-, intermediate-, and low-affinity receptors on lymphocytes, promoting secondary cytokine release, which recruits and activates additional cell types.⁸ Symptoms of toxicity occurring several hours after IL-2 administration are believed to result from the immunopathology caused by this widespread cell activation, and correspond with the release of secondary cytokines in the circulation.^{8,9} Secondary cytokine release and subsequent systemic toxicities may be reduced if selected cellular subsets are activated. One way to achieve this may be to administer lower doses of IL-2. Nanomolar concentrations of IL-2 can stimulate cell populations with high-affinity IL-2 receptors, such as natural killer cells and pre-activated T and B cells, but will not stimulate lymphocytes with lower-affinity IL-2 receptors.⁹ Lymphocyte proliferation is duration- and dose-dependent, with prolonged therapy producing more pronounced effects.^{8–11} Therefore, low-dose IL-2 administered over an extended period of time is an attractive alternative to the administration of high-dose IL-2 for shorter periods, as systemic toxicities may be minimized by selective expansion of T-cell populations activated against defined antigens.

Studies of single-agent low-dose IL-2 regimens have shown minimal clinical toxicity in patients with metastatic

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melanoma and renal cell carcinoma; however, objective clinical responses to these regimens have also been modest.^{9,12–16} Given the role of low-dose IL-2 therapy in promoting the proliferation of pre-activated antigen-specific T cells, the best use of low-dose IL-2 may be as a systemic adjuvant administered in combination with experimental cancer vaccines.⁸

In three clinical studies at our institution, low-dose IL-2 therapy (3×10^6 IU/m²/d) was paired with melanoma vaccines comprising peptide-pulsed dendritic cells, autologous tumor cells administered with GM-CSF, or synthetic peptides administered with GM-CSF. As IL-2 is a potent modulator of the immune system, and the antigens used in the vaccines were identical or similar to a portion of proteins expressed in normal tissues, antitumor immunity in conjunction with autoimmunity could have occurred. Antitumor responses may be considered a form of intentional autoimmunity. However, the combination of antitumor vaccines and low-dose IL-2 can lead to other autoimmune reactions. These reactions and the damage resulting from them are important to evaluate. Here, we review the clinical and laboratory data from 93 patients enrolled in three clinical studies to assess the occurrence of visual or ocular changes, vitiligo, thyroiditis, and diabetes associated with the administration of melanoma vaccines in conjunction with low-dose IL-2.

METHODS

Clinical Studies

Approval for the clinical studies was obtained from the University of Virginia Human Investigation Committee (HIC #7621, 8515, 8577) and the FDA (IND #7593, 8932). Informed consent was obtained from all enrolled patients. All patients were treated in an outpatient setting at the University of Virginia Cancer Center or General Clinical Research Center.

A total of 93 patients were enrolled, with 2 patients enrolling sequentially in two of these studies ($n = 95$). All patients were cytologically or histologically diagnosed with AJCC resected stage IIB, III, or IV melanoma, or stage IV melanoma with measurable disease. All patients had ECOG performance status of 0 or 1. Eligibility requirements included normal hematologic parameters and adequate liver and renal function. Criteria for exclusion included treatment with cytotoxic chemotherapy or radiation within the preceding 4 weeks; severe autoimmune disease, including active connective tissue diseases requiring medication; active hyperthyroidism; or uncontrolled diabetes. Patients requiring corticosteroids or those with significant cardiac disease were also ineligible.

Vaccines

Mel 31

Patients were randomized to one of two treatment groups. Patients on Arm 2 were to receive six vaccinations comprising 100 μ g each of the following peptides: YLEPGPVT (gp100₂₈₀₋₂₈₈), ALLAVGATK (gp100₁₇₋₂₅), YMDGTMSQV (tyrosinase₃₆₉₋₃₇₇, with post-translational change of N \rightarrow D at 371), and DAEKSDICTDEY (tyrosinase₂₄₀₋₂₅₁ with substitution of S for C at 244) plus 190 μ g of a tetanus toxoid peptide

(AQYIKANSKFIGITEL) and 225 μ g GM-CSF (Leukomax, Schering-Plough Research Institute, Kenilworth, NJ, or Leukine, Berlex, Seattle, WA) in Montanide ISA-51 adjuvant (Seppic Inc, Paris, France/Fairfield, NJ).

Patients on Arm 1 were vaccinated with monocyte-derived dendritic cells pulsed with the same peptides described above. For both arms, the vaccines were prepared and administered on days 1, 8, 15, 29, 36, and 43, as previously described.^{17,18} All patients self-administered a single subcutaneous (s.c.) injection of 3×10^6 IU/m²/d of recombinant human IL-2 (Proleukin [aldesleukin], Chiron Corp, Emeryville, CA) daily for 6 weeks (days 8–49).

Mel 36

All patients were to receive the peptide-based vaccine (100–200 μ g per peptide) plus 225 μ g GM-CSF, which was prepared and administered as described for Mel 31.^{18,19} Patients were randomized to self-administer a single s.c. injection of 3×10^6 IU/m²/d of IL-2 daily for 6 weeks, beginning either at day 8 or at day 29 of vaccine administration.

Mel 37

All patients were to receive 0.2 to 2.0×10^7 irradiated autologous tumor cells and 225 to 450 μ g GM-CSF in Montanide ISA-51 adjuvant, administered intradermally. Low-dose IL-2, 3.0×10^6 IU/m²/d, was administered s.c. for 6 weeks (days 8–49) (manuscripts submitted).

Toxicity Assessment and Data Analysis

Physical examinations and toxicity screening, based upon the NCI Common Toxicity Criteria v2.0 (CTC), were completed once before study entry and weekly during the course of vaccination. The weekly assessments were based on a daily symptom diary kept by patients, which were reviewed by interview with a study nurse or physician at each visit. A reported grade III or IV toxicity attributed to IL-2 resulted in a delay of IL-2 until the toxicity resolved to baseline or termination of therapy. Patients eligible to continue IL-2 therapy after their toxicity resolved received a 25% reduction of IL-2 for the subsequent doses. If the toxicity did not resolve to baseline, treatment with IL-2 was terminated. All available clinical data were reviewed (range 1 month to 4 years).

Peripheral venous blood samples were drawn weekly and analyzed using established standards at the University of Virginia Clinical Laboratory. Normal laboratory values were as follows: glucose, 70 to 105 mg/dL, with critical values of less than 40 mg/dL and more than 700 mg/dL; thyroid-stimulating hormone (TSH), 0.4 to 6.0 μ IU/mL; thyroxine (total T4, tT4), 4.5 to 10.9 μ g/dL; and free thyroxine (free T4, fT4), 0.8 to 1.8 ng/dL. Non-fasting serum glucose was measured at each study visit. Three patients did not receive treatment, one of whom had been enrolled in two trials, and were excluded from this analysis ($n = 91$). Patients were classified as evaluable for thyroid function if at least one pretreatment measurement and one measurement during treatment with IL-2 were documented and if they were not receiving medication for hypothyroidism prior to study entry ($n = 55$). Routine thyroid function screening was not required in the original Mel 31 protocol but was incorporated after the study was opened to enrollment.

Assessment of Immune Response to Vaccination

T-cell responses against peptides or autologous tumor cells were evaluated for IFN- γ release using an ELISPOT assay, as described previously.^{18,19}

RESULTS

Of 93 patients in three studies, 24 experienced one or more of the toxicities described below. The demographics of the patients experiencing the described toxicities are presented in Table 1. The toxicities were distributed through the studies (Table 2).

Visual/Ocular Toxicity

Four patients (4%) had ocular or conjunctival symptoms noted after treatment. These included conjunctival irritation caused by a foreign particle in the upper eyelid (VMM276), a complaint of fluttering of the left eye for several days after vaccine 5 (VMM193), a complaint of decreased visual acuity (VMM344), and redness in one eye for 4 to 5 weeks after the second vaccine (VMM220). All of these patients were formally evaluated for visual acuity and retinal changes weekly and at the time the symptoms were noted. Visual acuity was unchanged and ophthalmoscopic exams were normal in all cases.

Vitiligo

Six patients (7%) noted vitiligo (Tables 2 and 3). In each case the development of vitiligo was asymptomatic. In two of the six cases, vitiligo first appeared after the completion of protocol therapy (VMM150 and VMM326). VMM150 was one of two patients who enrolled in two studies, Mel 31 followed by Mel 37. This patient had intervening biochemotherapy after vaccination before the vitiligo was evident, which may have contributed to the vitiligo. The biochemotherapy was administered after the Mel 31 study but prior to entering the Mel 37 study. Vitiligo was noted over most of the skin of the chest and neck and also over a subcutaneous tumor nodule in the right arm that regressed completely after appearance of the vitiligo. In the second patient (VMM326), vitiligo was noted approximately 4 months after administration of the last vaccine; however, no intervening therapy was received. Therefore, the vitiligo was probably a result of the vaccines and IL-2 therapy.

Another patient (VMM214) received biochemotherapy, IFN therapy, and an autologous tumor cell vaccine prior to enrolling on the Mel 31 trial. Vitiligo was noted on the patient's arms, shoulders, and neck prior to vaccination on Mel 31. This patient went on to develop dramatic vitiligo with

TABLE 1. Patient Demographics

Toxicity	n	Males	Age (years)		
			Min	Max	Mean
Visual/ocular	4	2	51	72	60
Vitiligo	6	4	36	82	53
Hyperglycemia	2	2	41	72	56
Thyroiditis	14	10	29	72	54

TABLE 2. Patients with Autoimmune Toxicities

Toxicity	VMM	Study	Arm
Visual/ocular	276	31	1
Visual/ocular	193	31	2
Visual/ocular	344	31	2
Vitiligo	214	31	2
Vitiligo	273	31	2
Vitiligo	378	31	2
Vitiligo	326	31	2
Vitiligo	352	37	NA
Vitiligo	150	31 & 37	2 & NA
Hyperglycemia	253	31	2
Thyroiditis	155	31	1
Thyroiditis	244	31	1
Thyroiditis	232	31	2
Thyroiditis	226	31	2
Thyroiditis	371	31	2
Thyroiditis	334	31	2
Thyroiditis	270	36	1
Thyroiditis	293	36	1
Thyroiditis	246	36	2
Thyroiditis	216	36	2
Thyroiditis	261	36	2
Thyroiditis	287	36	2
Thyroiditis	366	37	NA
Visual/ocular, hyperglycemia, thyroiditis	220	36	1

nearly total body depigmentation observed during the course of vaccination. The last therapy this patient received was more than 9 months prior to enrollment on Mel 31. Thus, the exacerbation of the vitiligo was likely due to the protocol therapy.

In one patient (VMM378) vitiligo was present on the arms for years prior to study entry. The extent of the preexisting vitiligo did not change, nor did any new vitiligo develop during biochemotherapy, which was initiated approximately 10 months prior to enrolling into the Mel 31 trial. However, approximately 5 to 6 months post-vaccination, new areas of vitiligo occurred on the chest, shoulders, and arms.

Two additional patients (VMM352 and VMM273) had preexisting vitiligo that increased after vaccination; one of these is shown in Figure 1 (VMM273). For VMM352, the affected area increased dramatically approximately 18 months post-enrollment. However, this patient had intervening biochemotherapy, which may have contributed to the exacerbation of the vitiligo.

Hyperglycemia

Two patients experienced grade IV hyperglycemia (see Table 2). VMM253 was an otherwise healthy patient with no prior history of diabetes. After three vaccines and 2.5 weeks of IL-2 therapy, he developed diabetic ketoacidosis with a serum glucose level of 1030 mg/dL (Fig. 2). This led to a change in his mental status and required hospitalization in the intensive care unit of his local hospital. This patient was taken off protocol, and his diabetes was managed with insulin. Within 8 weeks of his hospitalization, the patient recovered to baseline

TABLE 3. Summary of Documented Vitiligo

Patient ID #	Study	Arm	Vitiligo Before Enrollment?	After Treatment	Received Biochemotherapy?
VMM150	Mel 31	2	No	Post-vaccination vitiligo	After vaccination; Legha regimen
VMM326	Mel 31	2	No	Post-vaccination vitiligo	None
VMM214	Mel 31	2	Yes	Dramatic expansion of vitiligo near the last vaccine, continuing to essentially complete total body vitiligo	Prior to vaccination; exact regimen unknown
VMM378	Mel 31	2	Yes	Additional vitiligo locations arising post-vaccination	Prior to vaccination; DTIC + interferon
VMM273	Mel 31	2	Yes	Expansion of pre-existing vitiligo areas	None
VMM352	Mel 37	1	Yes	Dramatic expansion of pre-existing vitiligo	After vaccination; DTIC + IL-2

status, was removed from insulin, and was euglycemic. This patient experienced a 40% regression of a mediastinal metastasis, followed by subsequent progression of his disease. Islet-cell reactive autoantibodies were not measured during his admission to the hospital or after recovery to baseline status.

The second case of grade IV hyperglycemia (VMM220) was a patient who ceased taking his prescribed diabetes medication upon study enrollment and had uncontrolled blood sugar (range 74–665 mg/dL) during the course of his treatment. The patient was instructed to take his diabetes medi-

cation and his blood sugar levels were then controlled, at which time vaccine and IL-2 administration resumed.

Thyroiditis

The NCI Common Toxicity Criteria v2.0 does not provide a grading scale for hyperthyroidism; therefore, we developed a grading scale for this adverse event (Table 4). Of 55 evaluable patients, 14 experienced thyroid abnormalities (25%), manifested by abnormal laboratory studies, including 2 cases of hypothyroidism (grade 2) and 13 cases of hyperthyroidism (10 grade 1, 3 grade 2) (see Table 2). All 14 patients had normal thyroid function at baseline as shown by normal laboratory values of TSH, free T4, and/or total T4. Antithyroid antibodies were not measured prior to enrollment. One patient (VMM 216) first experienced hyperthyroidism and later developed hypothyroidism and was counted once in the overall analysis. Figure 3 shows laboratory data representative of the hyperthyroid patients. These abnormalities often resolved within several weeks of stopping IL-2, and patients became euthyroid without the need for medications.

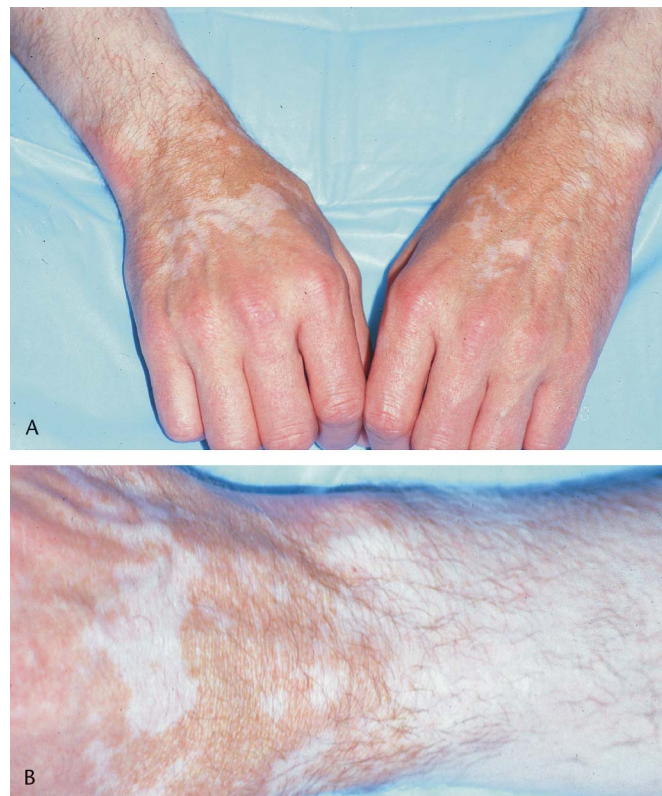


FIGURE 1. Vitiligo. A, The hands of a patient treated on Mel 31. B, Enlarged photo of the right hand shown in A.

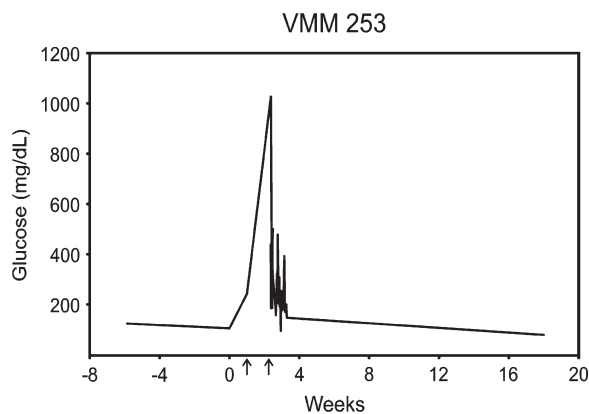


FIGURE 2. IL-2-induced hyperglycemia. After three vaccines and 2.5 weeks of receiving IL-2 (arrows), patient VMM253 unexpectedly developed diabetic ketoacidosis with a serum glucose level of 1,030 mg/dL. Within 8 weeks of his hospitalization, he was removed from insulin and was euglycemic.

TABLE 4. Grading Scale for Hyperthyroidism

Grade	Description of Hyperthyroidism
0	Absent
1	Asymptomatic. TSH depressed, free T4 +/- or free T3 elevated. No therapy given.
2	Symptomatic or treatment given
3	Patient hospitalized for manifestations of hyperthyroidism
4	Thyroid storm

The NCI Common Toxicity Criteria v2.0 does not have a grading scale for hyperthyroidism. To evaluate the severity of the hyperthyroidism experienced by patients enrolled in these trials, we developed the grading scale shown here.

However, at least four patients required medication. Two patients were hyperthyroid and were treated with methimazole (VMM226) or propranolol (VMM261), and one patient (VMM293) was hypothyroid and was treated with levothyroxine. The remaining patient (VMM216) was classified as having hyperthyroidism and his IL-2 was discontinued. He then developed hypothyroidism, which required treatment with levothyroxine.

Antibody tests were not routinely completed on all hyperthyroid patients; however, in three of the seven patients tested, antithyroid and antimicrobial antibodies were detected. The overall adverse events experienced by the patients with hyperthyroidism were not significantly different from those experienced by the patients who had normal thyroid function (data not shown).

Immunologic and Clinical Outcomes

Data on immune response and clinical outcome are given in Table 5. For Mel 31, 3 of the 26 patients enrolled had clinical responses,¹⁸ one of whom experienced vitiligo (VMM220; see Tables 2 and 3). For Mel 36, 86% of patients with autoimmune toxicities remained alive with no evidence of disease at 4.5 years, which compares favorably to the overall survival of approximately 65% for patients with stage IIB melanoma at 5 years.²⁰ For Mel 37, no clinical responses were reported in patients experiencing autoimmune toxicities.

DISCUSSION

Single-agent low-dose IL-2 regimens have been explored as alternatives to high-dose IL-2 therapy with the hope of minimizing toxicity. Unfortunately, the clinical response rate against melanoma of these low-dose regimens has been modest at best. However, as low-dose IL-2 may expand T-cell populations activated against specific tumor antigens, another clinical use may be the administration of this reagent in combination with specific antigen stimulators.

In three clinical studies, we paired different melanoma vaccine therapies with a daily regimen of low-dose IL-2. For the Mel 31 and Mel 36 studies, complete efficacy and safety data are presented elsewhere.^{18,19} Autoimmune reactions were important to evaluate separately as part of the safety analyses because the antigens used in the vaccines are identical or similar to a portion of proteins expressed in normal tissues, and IL-2 has been shown to augment immune responses. We

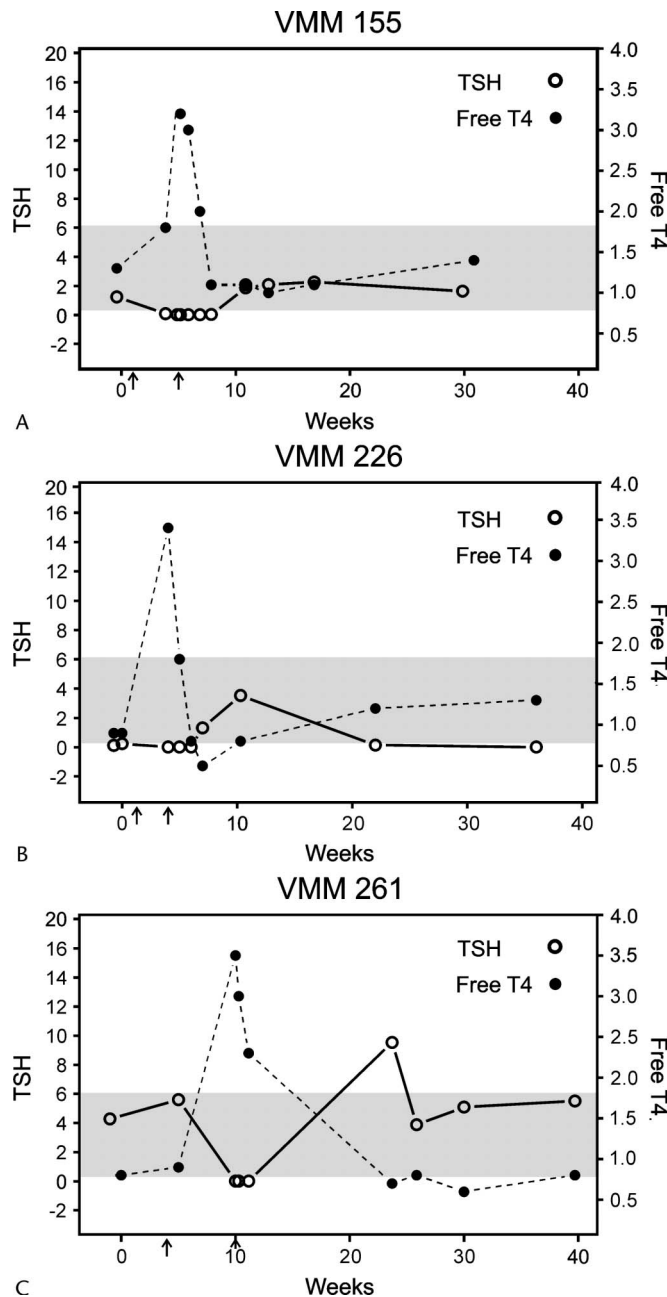


FIGURE 3. IL-2-induced hyperthyroidism. Plots representative of the patients experiencing hyperthyroidism. The shaded areas indicate the range of normal values for TSH and free T4 measurements. The arrows indicate the duration of each patient's IL-2 therapy.

reviewed the clinical and laboratory data from 91 patients to assess the occurrence of autoimmune toxicities associated with the administration of melanoma vaccines in conjunction with IL-2.

Visual/Ocular Toxicity

One of the major theoretical risks patients assume upon enrollment in a trial of a vaccine using melanoma-associated

TABLE 5. Immune Response and Clinical Outcomes Data for Patients with Autoimmune Toxicities

Study	n	Stage (N)	Immune Response Detected	Clinical Response		Median Follow-Up (years)	Alive
				CR/PR	SD		
Mel 31 ¹⁸	15	Advanced Stage III (2) or Stage IV (13)	5/10 (50%)	1/15 (7%)	3/15 (20%)	3.7	3/15 (20%)
Mel 36 ¹⁹	7	Resected Stage IIB (0), Stage III (6), or Stage IV (1)	5/7 (71%)	NA	NA	4.4	6/7 (86%)
Mel 37	3	Resected Stage III (1) Advanced Stage IV (1)	0/3 (0%)	0/3 (0%)	1/3 (33%)	NA	0/3 (0%)

CR, complete response; PR, partial response; SD, stable disease.

antigens is the potential development of melanoma-associated retinopathy (MAR). MAR is thought to be mediated by auto-antibodies directed against tumor antigens cross-reacting with retinal proteins, resulting in the sudden onset of night blindness, light sensations, and loss of visual acuity.^{21,22}

To date, there have been no published reports of MAR occurring in association with peptide-based vaccines.^{18,23–25} However, the administration of IL-2 in conjunction with melanoma vaccines could trigger this type of autoimmune response, if the vaccines contain proteins or peptides derived from proteins that are also found in the retinal pigment epithelium (eg, gp100 and tyrosinase). All patients enrolled in Mel 31 and Mel 36 had tumors that were positive for gp100 and/or tyrosinase expression, and at least 64% of the tumor cell preparations in the Mel 37 trial also expressed at least one of these proteins.

The majority of patients in Mel 31 (Arm 2)¹⁸ and Mel 36 (Arms 1 and 2)¹⁹ developed T-cell responses against the gp100 and tyrosinase vaccine components; however, none of these patients experienced MAR. Thus, even where tyrosinase- and/or gp100-specific T cells were present, no cases of MAR occurred.

Other visual disturbances or ocular toxicities were reported in four patients enrolled in the Mel 31 and Mel 36 studies receiving vaccine or IL-2 therapy. One event was determined to be unrelated to treatment. The other three warranted further examination, yet no changes in visual acuity or retinal pigmentation were detected. Thus, overall, visual and ocular toxicity may be more of a theoretical concern with vaccines combined with low-dose IL-2.

Vitiligo

The occurrence of vitiligo coincident with regressions of melanoma illustrates another possible implication of autoimmunity against cells of melanocytic lineage.²⁶ Most occurrences of vitiligo are limited to the skin surrounding the regressing melanoma; however, it can also occur systemically. In these cases, the loss of skin and hair pigment can be striking but is not a cause of morbidity or mortality. All six cases (7% [95% CI 2–14%]) presented here experienced systemic vitiligo, with some being more dramatic than others.

Vitiligo is often the result of a specific autoimmunity to antigens administered in vaccine preparations and has been reported with numerous other vaccination regimens.^{27–29} Rosenberg and White reported the occurrence of vitiligo in 11 of 74 (15% [95% CI 8–25%]) patients treated with high-dose IL-2 therapy²⁹ and Wolkenstein et al reported a rate of 4 of 25 (16% [95% CI 5–36%]) patients treated with low-dose IL-2.³⁰ Our observed rate was lower; this may be due in part to differences in treatment regimens. However, with the described treatment regimens, we cannot discern whether the rate of occurrence is attributable, wholly or in part, to the concurrent administration of IL-2.

Hyperglycemia

Soni et al previously reported one case of diabetes mellitus induced by a dose of IL-2 (1.5 × 10⁶ IU/m²/d), lower than that administered in the trials presented here.³¹ In the present report, non-fasting serum glucose levels were measured weekly during the vaccine regimen and at each follow-up visit. Two patients experienced grade IV hyperglycemia. One patient had no prior history of diabetes, and the attribution of his insulinitis to the IL-2 regimen is clear. Similar to what has previously been described,³¹ hyperglycemia reversed once IL-2 was discontinued. The second patient ceased taking his prescribed diabetes medication upon study enrollment; therefore, the attribution of his insulinitis to the IL-2 regimen is less apparent. IL-2 therapy may have exacerbated his condition; however, his diabetes remained controlled once he resumed taking his medication, despite resumption of IL-2 therapy. Presumably, the observed effects were due to transient insulinitis.

As evidenced by these two cases, glucose levels should be closely monitored in patients receiving low-dose IL-2. We propose monitoring glucose levels after fasting. In addition, due to the rapid rise in serum glucose levels observed in these two patients, the most effective method of evaluation may be an in-home analysis completed by patients on a daily basis. Patients should be fully informed that IL-2-induced diabetes is a potential side effect, because we do not know whether pancreatic function will normalize in all patients upon cessation of IL-2 therapy.

Thyroiditis

Exacerbation of thyroiditis has been reported to occur with low- and high-dose IL-2 therapy.^{32,33} This exacerbation is presumed to be an autoimmune thyroiditis, which could become an irreversible Hashimoto's thyroiditis. Fourteen of 55 (25%) evaluable patients had detectable thyroid abnormalities (95% CI 15–39%). This rate of thyroiditis may be underestimated because thyroid function tests were not conducted at regular intervals at the onset of the Mel 31 study. However, a more rigorous assessment of thyroid function was incorporated in later clinical studies involving low-dose IL-2. Schwartzentruber et al³⁴ and Krouse et al³⁵ reported similar rates of thyroid dysfunction in 35 of 107 (33% [95% CI 24–42%]) and 54 of 188 (29% [95% CI 22–36%]) patients treated with IL-2-based immunotherapy, respectively.

Of the 14 patients with thyroid abnormalities reported here, 7 were evaluated for antithyroid and antimicrosomal antibodies. Three of these patients developed antithyroid and antimicrosomal antibodies, consistent with an autoimmune thyroiditis. Therefore, patients who are being screened for participation in clinical studies incorporating IL-2 therapy should be tested for thyroid abnormalities and possibly excluded if they present with these abnormal laboratory values. In addition, patients who receive IL-2 therapy should be monitored closely for thyroid-related toxicities, a practice we now incorporate into clinical trials involving IL-2. Patients should also be informed of the possibility of compromised thyroid function and the potential of conversion to being hypothyroid long-term, including a dependency on thyroid replacement medication.

Immunologic and Clinical Outcome

Table 5 summarizes the immune response and clinical outcome data for the 24 patients enrolled in Mel 31, 36, or 37 who experienced an autoimmune toxicity. In several patients who experienced an autoimmune toxicity, immune responses to vaccine components were not detectable (see Table 5). Thus, these toxicities did not correlate well with peptide-specific T-cell reactivity. Larger sample sizes are needed to assess the relationship, if any, between the development of an autoimmune reaction after vaccination and clinical outcome.

The majority of autoimmune toxicities experienced by the patients enrolled in these three clinical trials affected one of a select number of organ systems. Most of these reactions were transient and well-tolerated and contributed minimally to morbidity. These results indicate that autoimmune toxicities related to IL-2 therapy should be monitored and evaluated when designing new cancer-related immunotherapies. In addition to the autoimmune toxicities discussed above, our patients are currently screened for the presence of antinuclear antibodies and rheumatoid factor levels prior to and during treatment. These tests should be included as part of the complete assessment of patients receiving immunologic therapies for cancer. Rigorous screening for autoimmune toxicities will allow for a more accurate determination of the rate of occurrence associated with IL-2 treatments.

In patients on other trials of peptide vaccines without administration of low-dose IL-2, we have not observed autoimmune toxicities at the frequencies reported here (data not shown). This suggests these autoimmune toxicities are

more likely to be attributable to the administration of low-dose IL-2 therapy than to the peptide vaccines. The evidence for autoimmune toxicities with low-dose IL-2 suggests it can aid in breaking tolerance to self-antigens, which often serve as targets in antitumor immune responses. A treatment regimen of low-dose IL-2, possibly with variation in timing and dosage, may be useful to administer with various types of targeted immunologic therapies for cancer.

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