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REVIEW ARTICLE

Vitiligo-Like Depigmentation in Patients With Stage III-IV Melanoma Receiving Immunotherapy and Its Association With Survival: A Systematic Review and Meta-Analysis

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ABSTRACT

Purpose
Vitiligo-like depigmentation in patients with melanoma may be associated with more favorable clinical outcome. We conducted a systematic review of patients with stage III to IV melanoma treated with immunotherapy to determine the cumulative incidence of vitiligo-like depigmentation and the prognostic value of vitiligo development on survival.

Methods
We systemically searched and selected all studies on melanoma immunotherapy that reported on autoimmune toxicity and/or vitiligo between 1995 and 2013. Methodologic quality of each study was appraised using adapted criteria for systematic reviews in prognostic studies. Random-effect models were used to calculate summary estimates of the cumulative incidence of vitiligo-like depigmentation across studies. The prognostic value of vitiligo-like depigmentation on survival outcome was assessed using random-effects Cox regression survival analyses.

Results
One hundred thirty-seven studies were identified comprising 139 treatment arms (11 general immune stimulation, 84 vaccine, 28 antibody-based, and 16 adoptive transfer) including a total of 5,737 patients. The overall cumulative incidence of vitiligo was 3.4% (95% CI, 2.5% to 4.5%). In 27 studies reporting individual patient data, vitiligo development was significantly associated with both progression-free-survival (hazard ratio [HR], 0.51; 95% CI, 0.32 to 0.82; $P < .005$) and overall survival (HR, 0.25; 95% CI, 0.10 to 0.61; $P < .003$), indicating that these patients have two to four times less risk of disease progression and death, respectively, compared with patients without vitiligo development.

Conclusion
Although vitiligo occurs only in a low percentage of patients with melanoma treated with immunotherapy, our findings suggest clear survival benefit in these patients. Awareness of vitiligo induction in patients with melanoma is important as an indicator of robust antimelanoma immunity and associated improved survival

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INTRODUCTION

Melanoma immunotherapy studies have shown variable success rates in inducing effective antimelanoma immune responses. The occurrence of immune-related adverse effects after melanoma immunotherapy has been associated with increased clinical efficacy.[1-6] Vitiligo-like depigmentation, also referred to as vitiligo, is a relatively harmless type of autoimmunity that can occur in patients with melanoma spontaneously or on immunotherapy. The depigmentation results from strong antimelanoma immunity that also targets healthy melanocytes, as a result of shared expression of melanocyte differentiation antigens. The incidence of depigmentation in patients with melanoma varies largely between immunotherapy studies.[7-10] A large, prospective, hospital-based, observational study showed a cumulative incidence of 2.8% of melanoma-associated vitiligo in 2,954 patients with melanoma of different stages regardless of treatment.[11] Importantly, vitiligo development in patients with stage III and IV melanoma was associated with tumor regression and prolonged survival in individual studies.[5,7,8,11-14] However, it is not clear to what extent these results can be extrapolated to all immunotherapy studies. Also, it is currently difficult to predict which patients respond to immunotherapeutic treatment. Present prognostic (bio)markers in melanoma are based on the American Joint

Committee on Cancer TNM staging system. These biomarkers include Breslow tumor thickness, presence of ulceration, extent of nodal involvement for primary cutaneous melanoma, site of distant metastases, and serum lactate dehydrogenase and are related to general disease progression and survival.[15,16] Response-predictive biomarkers to immunotherapy are scarce. A prognostic factor to evaluate outcome in patients with melanoma receiving immunotherapy is needed. Vitiligo development may be useful as a simple visible clinical parameter of antimelanoma immunity and clinical response. Therefore, we conducted a systematic review of patients with stage III or IV melanoma treated with immunotherapy to determine the cumulative incidence of vitiligo-like depigmentation development and the prognostic value of vitiligo development on progression-free survival (PFS) and overall survival (OS).

PATIENTS AND METHODS

The search strategy, review process, selection of studies, data extraction, and methodologic quality assessment are described in the Appendix (online only). The complete MEDLINE search can be found in the Data Supplement.

Statistical Analysis

To calculate the pooled cumulative incidence of vitiligo (in percentage), one has to take into account that the true incidence per study may vary as a result of clinical and methodologic differences between studies. Therefore, random-effects models were used to meta-analyze the (logit transformed) percentage of patients developing vitiligo in each study. These models take into account the precision by which the percentage has been estimated in each study using the binomial distribution (ie, weighted average with larger studies receiving more weight) and incorporate any additional variability beyond chance that exists between studies (ie, random-effects approach). Results are presented as summary estimates of vitiligo as percentages together with random-effect 95% CIs. Several study characteristics were added to the basic model to examine whether the percentage of vitiligo differed between subgroups of studies. The following characteristics were examined: type of intervention/immunotherapy, studies with different methodologic quality, and studies reporting autoimmune toxicity but not mentioning vitiligo specifically. The nonlinear mixed models procedure (PROC NLMIXED) of SAS 9.1 (SAS Institute, Cary, NC) was used to estimate the random-effects pooled percentage of vitiligo. $P < .05$ was considered statistically significant.

In the second systematic review, we used studies reporting individual patient data (IPD) on melanoma immunotherapy treatment and survival. Using these IPD, we constructed univariable Kaplan-Meier survival curves for OS and PFS and stratified for patients with and without vitiligo development (log-rank comparison).[17]

The prognostic value of vitiligo was analyzed by multivariable survival analysis (Cox proportional hazards regression model) computing hazard ratios (HRs) and 95% CIs using a random-effects model adjusted for age and sex for both PFS and OS. PFS in time was extended by 1 day (+1/30.25) to include patients with PFS 0 at time of clinical evaluation who would be otherwise excluded for the analysis. The presence of heterogeneity was assessed by performing the Cochran Q test. Statistical analyses were performed using the R environment (http://www.r-project.org; release version 2.15.1) and the SPSS statistical package (version 18.0; SPSS, Chicago, IL). Values of 95% CIs were used for all analyses. $P < .05$ was considered significant.

To examine whether the appearance of vitiligo was associated with the induction of melanoma-specific immunity, we have analyzed all studies included in the survival analyses and created a 2 × 2 table with the number of patients with vitiligo induction (+/–) and melanoma-specific immunity (+/–) on treatment. To this end, data were extracted on the type of immune activation measured, the number of patients monitored for antimelanoma immunity, the number of patients who experienced activation of melanoma-specific immunity, and the number of patients with vitiligo who experienced activation of melanoma-specific immunity. Only studies that reported immunomonitoring on the patient level and only patients who completed therapy were included for analysis. Because multiple types of immune responses and techniques per study were used, we decided to include the immune assay with the highest number of patients analyzed. In case the numbers of analyzed patients were similar in all assays, we choose systemic T-cell response analyses as the best outcome of all immunomonitoring. The resulting 2 × 2 table was analyzed using the χ^2 test (two-tailed).

RESULTS

Results of the Electronic Literature Search

The combined searches retrieved 3,710 records from MEDLINE and EMBASE databases (Fig 1). On the basis of the eligibility criteria for title and abstract, 533 studies were selected for full-text screening. Reasons for exclusion in the primary selection of abstracts are listed in Fig 1. Lack of evaluation of any type of autoimmune toxicity and/or vitiligo was the most frequent exclusion reason in the full-text screening. In total, 137 studies met the inclusion criteria.

Description of Included Studies

The characteristics of the 137 studies comprising 139 treatment arms in total are summarized in Table 1 (Hodi et al[18] reported a three-arm study). Details of the included studies are presented in the Data Supplement, Table S2.[1,4,5,6,12,13,19-147] All studies, except for one, were published in English. The study selection contained 52 phase I studies (37%), 45 phase I/II studies (32%), 25 phase II studies (18%), four phase III studies (3%), four cohort studies (3%), five retrospective studies (4%), and four case series (3%). Half of the studies involved patients with stage III or IV melanoma (n = 66, 48%), and 65 studies (47%) involved patients with stage IV melanoma only. Five studies (4%) were performed in patients with resected stage III or IV melanoma, and three studies (2%) were performed in patients with stage III melanoma only. The median number of patients per study was 19 (interquartile range, 12 to 35 patients). Most of the studies (87%) were phase I or II trials, reporting on the toxicity of a new immunotherapeutic strategy and clinical outcome. Outcome criteria reported were RECIST (n = 42), (modified) WHO (n = 22), other criteria (n = 16), and unknown criteria (n = 49). Of the 139 included treatment arms, 98 (70%) reported on the presence or absence of vitiligo-like depigmentation on treatment. The other included studies only reported the presence or absence of signs of autoimmune toxicity and did not mention vitiligo specifically. Only 10 (7%) of 137 studies reported a complete skin examination to screen for vitiligo at baseline in the Methods section. Only one of these 10 studies described the use of Wood's light examination. All other studies reported newly developed vitiligo on treatment in the Results or Discussion section, often as an adverse effect and without any specification; the size of the lesion or by whom vitiligo was assessed was not described in any study. No study mentioned the involvement of a consulting dermatologist.

Quality Assessment

The risk of bias assessment resulted in 43 (31%) of 139 studies with a low risk of bias. Thirty-eight studies (27%) had an unclear risk of bias, and 58 studies (42%) had a high risk of bias. Studies with low risk of bias clearly indicated the total number of patients completing therapy, loss to follow-up, patient characteristics, possible confounders, and presence or absence of vitiligo development,

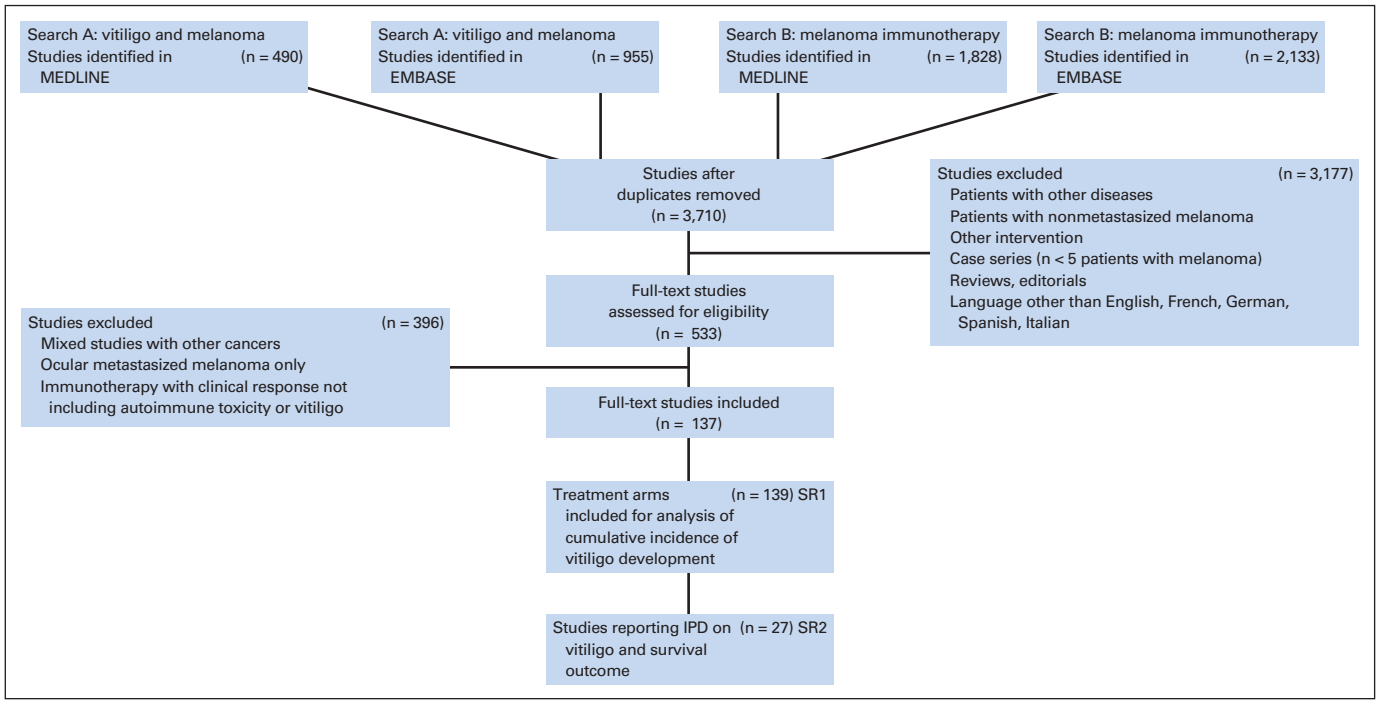

Fig 1. Flowchart of identification, selection, and analysis of included studies. IPD, individual patient data; SR1, systematic review 1; SR2, systematic review 2.

whereas studies with high risk of bias often did not report on the presence or absence of vitiligo but only on autoimmunity in general. For the second systematic review, 15 (56%) of 27 studies included in the survival meta-analysis had a low risk of bias, eight (30%) had an unclear risk of bias, and four (15%) had a high risk of bias. The summary of risk of bias assessment per domain is depicted in Figure 2. The assessment of methodologic quality per study is documented in the Data Supplement.

Table 1. Characteristics of Included Studies (Summary)

Melanoma Immunotherapy Treatment Arms and Study Design	No. of Studies	No. of Studies		No. of Patients per Study		No. of Patients With Vitiligo	
		Autoimmunity Only	Vitiligo	Median	Interquartile Range	Median	Interquartile Range
Immune stimulation	11	3	8	27	16-71	1	0-9
Phase I	2						
Phase I/II	2						
Phase II	4						
Phase III	1						
Retrospective cohort	2						
Vaccines	84	23	61	16	11-26	1	0-2
Phase I	38						
Phase I/II	33						
Phase II	4						
Phase III	1						
Case series	3						
Antibody	28	11	17	45	23-117	1	0-3
Phase I	5						
Phase I/II	8						
Phase II	6						
Phase III	2						
Cohort	4						
Retrospective	3						
Adoptive transfer	16	4	12	14	10-20	1	0-1
Phase I	7						
Phase I/II	2						
Phase II	6						
Case series	1						

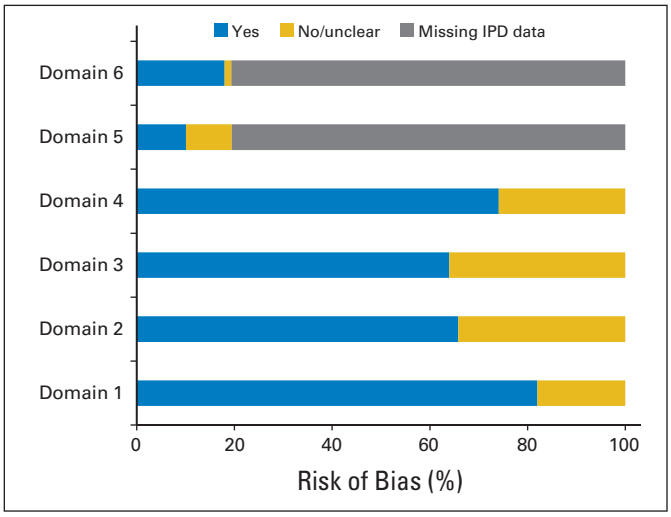

Fig 2. Summary risk of bias assessment per domain. Domains: (1) the study sample represents the population of interest; (2) loss to follow-up is not associated with key characteristics; (3) the prognostic factor is adequately measured in study participants; (4) important potential confounders are accounted for; (5) the outcomes of interest (survival) are adequately measured in the study; and (6) the statistical analysis is appropriate for the design of the study. IPD, individual patient data.

Vitiligo Development

Overall, in 85 of 139 treatment arms, patients with melanoma developed vitiligo-like depigmentation on immunotherapy. Three hundred four of the 5,737 patients included developed vitiligo. The summary estimate of the cumulative incidence of vitiligo-like depigmentation was 3.4% (95% CI, 2.49% to 4.53%; Fig 3). Excluding five retrospective studies, the cumulative incidence of vitiligo was 3.6% (95% CI, 2.64% to 4.78%) In 98 studies specifically reporting on vitiligo, excluding the studies that only found other autoimmune adverse events (not vitiligo), a higher cumulative incidence of vitiligo of 6.0% (95% CI, 4.72% to 7.72%) was found. Eleven studies used general immune-stimulation strategies such as including interferon alfa and interleukin-2, which resulted in a cumulative incidence of vitiligo of 4.4% (95% CI, 1.83% to 10.31%). Eighty-four vaccination studies were included, using vaccines based on dendritic cells, tumor cells, tumor antigenic peptides, and/or gene transfers, yielding a cumulative incidence of 3.8% vitiligo (95% CI, 2.63% to 5.46%). Twenty-eight studies used CTLA-4 blockade (ipilimumab or tremelimumab) or anti-PD1 antibodies (nivolumab or lambrolizumab), resulting in a cumulative vitiligo incidence of 2.0% (95% CI, 1.11% to 3.48%). Adoptive transfer of tumor-infiltrating lymphocytes was done in 16 studies, yielding a cumulative vitiligo incidence of 6.3% (95% CI, 2.76% to 12.44%).

A subgroup analysis on the cumulative incidence of vitiligo per risk of bias group yielded a vitiligo incidence rate of 6.8% (95% CI, 4.19% to 9.71%) in studies with a low risk of bias. Studies with a high risk of bias showed a cumulative incidence of vitiligo of 0.9% (95% CI, 0.53% to 1.58%). Finally, studies with an unclear risk of bias had a cumulative incidence of vitiligo of 6.5% (95% CI, 4.27% to 9.68%; Fig 3).

Survival Analyses

Twenty-seven studies included IPD on vitiligo (present or absent) and PFS and/or OS. These studies involved 418 patients with stage III or IV melanoma who completed immunotherapy treatment. Only one of the 27 studies described vitiligo screening at baseline in the

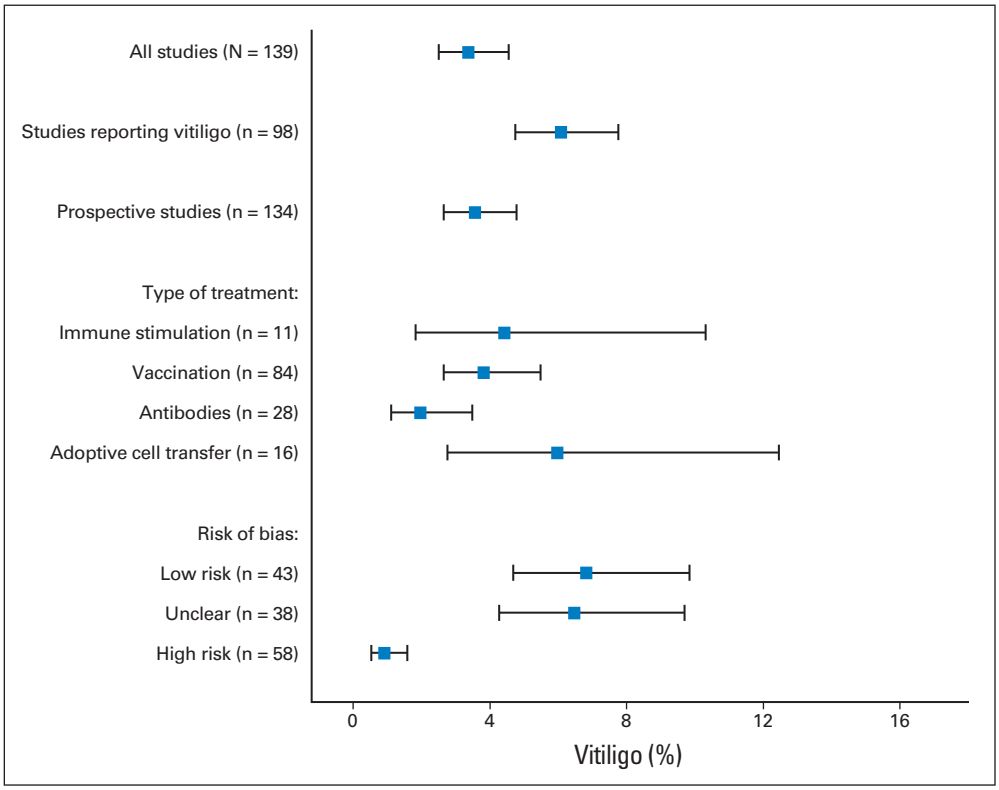

Fig 3. Summary estimates and 95% CIs of cumulative vitiligo incidence overall and by subgroups of studies.

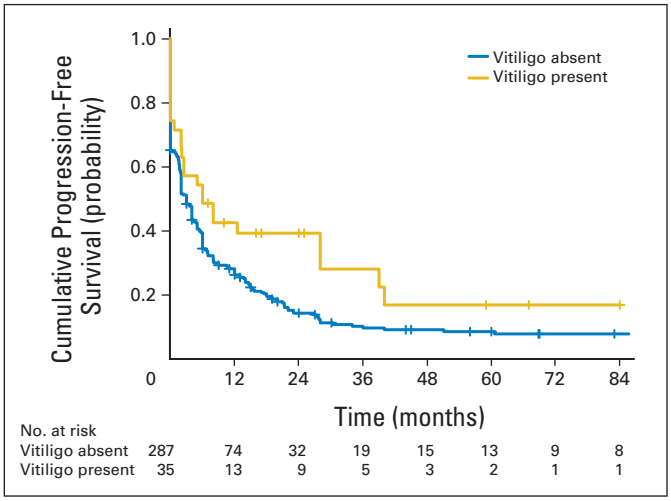

Fig 4. Progression-free survival in 322 patients receiving immunotherapy from 22 studies.

Methods section.[36] The remaining 26 studies reported vitiligo development on therapy as an adverse effect in the Results or Discussion section. Four of the 27 studies also reported an occasional patient with existing depigmentation that increased in severity during therapy.[42,69,71,101] Univariable survival analysis (log-rank based comparison of Kaplan-Meier curves) on vitiligo and PFS showed a significant survival benefit for the patients with vitiligo ($P < .031$; Fig 4). The Kaplan-Meier curve of vitiligo and OS showed a significant difference in OS between vitiligo and nonvitiligo patients ($P < .024$; Fig 5). No significant statistical heterogeneity was present among included studies (Q statistic $P = .4$; $I^2 = 0.0\%$). Therefore we pooled all IPD and used a random effects Cox proportional hazards model (with study number as random effects) to estimate the overall effect of vitiligo on PFS and OS. The HR of the association between vitiligo and PFS adjusted for age and sex was 0.51 (95% CI, 0.32 to 0.82; $P < .005$), indicating that patients who develop vitiligo during melanoma immunotherapy have two times less risk of disease progression compared with patients without vitiligo. The Cox regression

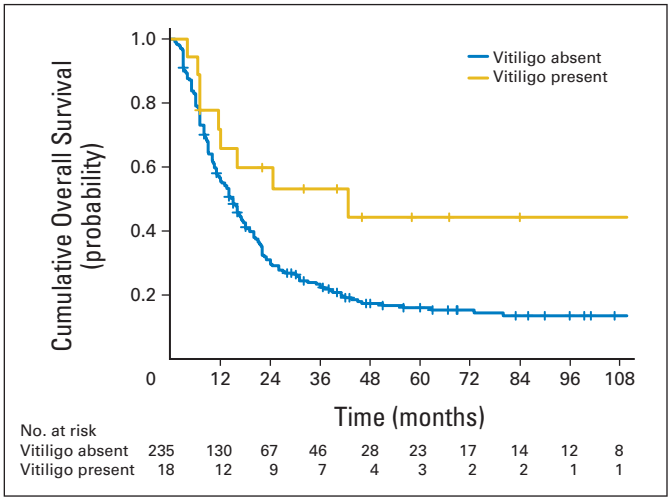

Fig 5. Overall survival in 253 patients receiving immunotherapy from 15 studies.

random-effects analysis on vitiligo and OS adjusted for age and sex resulted in an HR of 0.25 (95% CI, 0.10 to 0.61; $P < .003$), indicating four times less risk of death in patients with vitiligo development compared with patients without vitiligo.

The results of the analysis of melanoma-specific immunity and vitiligo induction on the individual patient level are presented in the Data Supplement. Statistical analysis of the 2×2 table resulted in a two-tailed $P = .0031$, implying that vitiligo development is significantly associated with melanoma-reactive immune activation.

DISCUSSION

In the present systematic review, all melanoma immunotherapy studies since 1995 were screened for vitiligo-like depigmentation in patients. We found a pooled cumulative incidence of vitiligo of 3.4% in 5,737 patients with stage III or IV melanoma on immunotherapy. Across the included types of immunotherapy, most cases of vitiligo (6.3%) were found on adoptive transfer of cytotoxic T-lymphocyte therapy. Also, a meta-analysis was performed with individual patient data on vitiligo development and its prognostic value for PFS and OS. Our review suggests that patients with melanoma with vitiligo have a two-fold decreased risk of disease progression and a four-fold decreased risk of death compared with patients without vitiligo development. This study indicates the significance of vitiligo as a clinical marker for effective antimelanoma immunity and clinical outcome after immunotherapy in patients with melanoma.

Little is known about the incidence of vitiligo in patients with melanoma and the relationship with clinical outcome in general, because most evidence comes from individual studies or case reports. In 1996, Rosenberg and White[9] prospectively evaluated vitiligo development in patients with melanoma treated with high-dose interleukin-2–based immunotherapy and found vitiligo in 15% of patients; patients with vitiligo had a response rate of 61% compared with 20% in nonvitiligo patients. A prospective Italian study showed a cumulative incidence of vitiligo of 5.4% in 738 patients with stage III melanoma treated with adjuvant interferon, which is comparable to the incidence we found.[11] The study also showed that vitiligo was associated with PFS and OS in patients with stage III and IV disease. Conversely, our previous study of patients with vitiligo showed that they had a decreased risk of developing melanoma during life, supporting the protective role of vitiligo against melanoma.[148]

The field of melanoma immunotherapy has evolved greatly in the last few decades. For this review, we screened all full texts describing any type of immunotherapy in patients with melanoma, because many studies did not report on autoimmune toxicity or vitiligo in the abstract. The majority of these studies did not report on autoimmune toxicity at all, some reported single autoimmune adverse effects, and even fewer reported on vitiligo development. In the last few years, with the introduction of anti–CTLA-4 antibody therapy, more attention has been paid to autoimmune toxicity and its characteristic spectrum of immune-related adverse events.[149] However, because vitiligo is usually a grade 1 toxicity and considered a mild adverse effect not requiring treatment, it is not always reported.

A careful appraisal of methodologic quality of all included studies was performed. One third of the studies to evaluate the incidence of vitiligo had a low risk of bias. Most studies were at an unclear or increased risk of bias as a result of no clear evaluation of vitiligo and

limited data on patient follow-up, patient drop-out, completion of treatment, and moment of clinical evaluation. Of the studies included in the meta-analysis on survival, 60% had a low risk of bias, which can be explained by clear IPD representation, a homogeneous patient population with mostly stage IV melanoma unresponsive to prior treatment, assuming considerable homogeneity in this review's population, and correct use of international standards for clinical evaluation such as RECIST and WHO criteria. We only included studies reporting on the presence or absence of vitiligo in the meta-analysis of PFS and OS, excluding studies with a high risk of bias reporting on autoimmune toxicity but not vitiligo.

Limitations of our analyses include combining survival and vitiligo development data across studies, using outcome definitions that are variable across studies. Importantly, the outcome parameter of vitiligo was of observational nature and based on the investigator's level of awareness of vitiligo because this was not an outcome parameter in any of the included studies. No randomized studies and only a few phase III studies were included in this review because of a lack of grade 1 or 2 autoimmune toxicity evaluations. All studies only describing the absence or presence of any autoimmune toxicity parameter other than vitiligo were defined as vitiligo negative. Therefore, we may have underestimated the incidence of vitiligo in this meta-analysis. In general, vitiligo may have been considered as a nonsevere autoimmune toxicity that was not necessary to report. Also, it is often not clear whether proper skin examinations for vitiligo development had been performed. Dermatologists are usually not involved in these studies, and it is unclear to what extent oncologists accurately diagnose vitiligo in patients with fair skin types. We recommend that all future prospective immunotherapy studies in patients with melanoma to include complete skin examinations by a dermatologist using a Wood's lamp to screen for vitiligo at baseline and other time points. A Wood's lamp is a hand-held fluorescent lamp emitting long-wave ultraviolet A light, which delineates areas of pigment loss.

The limited life span of patients with end-stage melanoma and their decreased functional immune system before entering an experimental study affect the successful induction of antitumor immunity, vitiligo induction, and survival, possibly underestimating the currently estimated vitiligo incidence.

We excluded studies combining several types of cancers to avoid interpretational problems. Less attention could be expected regarding vitiligo awareness and detection in patients with other types of cancer because vitiligo is uncommon in nonmelanoma patients. Although they included an interesting patient population, we also excluded combined chemotherapy-immunotherapy studies, because the clinical response could not be solely explained by the immunotherapeutic strategy and associations between vitiligo and survival would not be based on immune activation only. We do not know the effect of prior therapy on vitiligo development, but because most studies were phase I or phase II, all included patients were heavily pretreated, so we expect this effect to be limited.

Altogether, the results of this systematic review show a favorable effect of vitiligo induction as a relevant clinical parameter in patients with end-stage melanoma receiving immunotherapy. Although vitiligo occurs in only a relatively low percentage of treated patients with melanoma, our findings suggest a clear survival benefit in these patients and association with induction of antimelanoma immunity. To draw better conclusions from vitiligo and autoimmunity development in general in immunotherapy studies, we recommend the use of the immune-related adverse events criteria and reporting vitiligo systematically on the IPD level (in addition to monitoring of immune responses) in future melanoma immunotherapy studies. More awareness of vitiligo induction in patients with melanoma by oncologists may contribute to better recognition of patients with effective antimelanoma immunity and may influence their treatment options and prognosis.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

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Final approval of manuscript: All authors

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Vitiligo-Like Depigmentation in Patients With Stage III-IV Melanoma Receiving Immunotherapy and Its Association With Survival: A Systematic Review and Meta-Analysis

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Appendix

Search Strategy

A medical librarian (J.L.) undertook a systematic search of the electronic bibliographic databases MEDLINE and EMBASE (OVID) from January 1, 1995 to July 2013, using both free-text words and index terms specific to each database (MeSH in MEDLINE). The search included an iterative process to refine the search strategy through adding search terms as new relevant citations were identified. Two search approaches were followed. In the first search (search A), we searched for " melanoma" and "vitiligo" and related synonyms. For vitiligo, these were depigmentation, hypopigmentation, leukoderma, halo naevus, halo nevi, halo naevi, poliosis, and various text words describing destruction of melanocytes or antimelanocyte (auto)immunity (Data Supplement Table S1). The second search (search B) served to find melanoma immunotherapy studies that only mentioned vitiligo in the full text and not in title, abstract, and index terms. Search B consisted of the following components: "melanoma"; "a methodological search filter for appropriate original studies," that is, including trials (also phase I and II and open- or off-label), prospective and retrospective studies, and studies analyzing survival and remission; and "immunotherapy (modalities)." The bibliographic records retrieved were downloaded and imported into Reference Manager software (version 12.0; Thomson Reuters, New York, NY) to deduplicate, store, and analyze the search results.

Types of Studies

Melanoma immunotherapy studies, clinical trials (phase I, II, and III), case series (n $\geq$ five evaluable patients), prospective cohort studies, and retrospective studies that reported on clinical response to therapy and evaluated toxicity, including any type of clinical autoimmune adverse effects (vitiligo, colitis, hepatitis, or hypophysitis) were considered for this review. Studies that did not report a toxicity evaluation and all studies in which the toxicity evaluation did not mention the presence or absence of any autoimmune events were excluded. The adverse events/autoimmune toxicity had to be described in the Methods, Results, or Discussion section. Only publications written in English, French, German, Italian, or Spanish were selected. Immunotherapy studies including patients with different cancer types and not just melanoma were excluded. Reviews, editorials, and conference abstracts were excluded.

Patients

Patients age 18 years or older with stage III or IV (as defined by the American Joint Committee on Cancer) melanoma and primary cutaneous melanoma were considered for this review. No restrictions regarding sex, ethnicity, or performance status were applied. Only patients who completed the treatment and were evaluated for clinical response (Landmark), as defined per individual immunotherapy study, were included.

Interventions

All systemic immunotherapeutic regimens (see included synonyms/search terms) in patients with melanoma based on inducing a cellular or humoral antimelanoma immune response were included. Types of immunotherapies were categorized as general immune stimulation or cytokine therapy (including all cytokines applied directly to patients; eg, interferon alfa, interleuking-2, granulocyte-macrophage colony-stimulating factor); vaccinations (including dendritic cell therapy, vaccines consisting of [manipulated] tumor cells, dendritic cell, and/or tumor antigenic peptides or proteins and combinations of vaccines and cytokine therapy); antibody therapy (including all therapy variants using monoclonal antibodies [mAbs] to induce immune stimulation; eg, anti–CTLA-4 mAb, anti-PD1 mAb); or adoptive cell transfer of tumor-infiltrating lymphocytes. Studies combining mAbs with vaccinations were categorized as mAb studies.[1,18,132] No restrictions were made with respect to dose, type, frequency, application, and duration of the immunotherapy regimen as long as the study end point was clearly stated. Combined chemotherapy-immunotherapy studies were excluded, except in case of pretreatment with nonmyeloablative lymphodepleting conditioning regimens as used in adoptive transfer studies.

Outcome Measures

Two main types of outcome were of interest in this review. In the first systematic review, the outcome was any autoimmune toxicity and/or vitiligo development during melanoma immunotherapy in patients who completed treatment. Positive vitiligo status was defined as the development of depigmentation or its synonyms leukoderma and hypopigmentation, the development of halo nevi, or the exacerbation of a pre-existent vitiligo at the end of study. A negative vitiligo status was defined as no vitiligo development reported in the text or the description in the text that no vitiligo or skin depigmentation occurred. The body surface affected by the depigmentation or how it was assessed was not extracted because this was not reported in most studies. Progression of an existing hypopigmentation/vitiligo lesion on therapy was included, because this is also a marker of activation of antimelanoma immunity. Studies reporting on other autoimmune toxicity or reporting no occurrence of autoimmune toxicity were defined as negative for vitiligo development to limit selection bias.

In the second systematic review, the outcomes were progression-free survival (PFS) and/or overall survival (OS), in months, from start of treatment in patients who did and did not develop vitiligo during immunotherapy. All studies from the first systematic review that reported individual patient data on the presence or absence of vitiligo and survival outcome (OS/PFS) were selected.

Review Procedures

Selection of studies. Eligibility was performed independently and in an unblinded standardized manner by three reviewers (H.-E.T., S.N.J., and R.M.L.). Double publications reporting on the same study at a later time point were retrieved and excluded from further analysis. Any disagreements between reviewers were resolved by consensus.

Data extraction. The data from the included studies were extracted by two independent reviewers (H.-E.T. and R.M.L.) using a tailored data extraction form, which was first validated in a pilot of 20 randomly selected studies and refined accordingly. For the first systematic review, information was extracted on characteristics of study participants, the number of patients who started therapy, the number of patients who completed therapy (Landmark), and melanoma stage (American Joint Committee on Cancer stage); the study design (clinical trial phase I, I/II combined, phase II, or phase III; case series with $\geq$ five evaluable patients; prospective cohort study; or retrospective study; if studies were not categorized for study phase, the authors assigned a study category based on the objectives and study design); type of immunotherapy and the duration of therapy until the evaluation for clinical response, as defined per individual immunotherapy study; and type of toxicity screening including autoimmune toxicity and/or vitiligo. For the second systematic review, additional data extraction was performed on mean age, sex, outcome criteria, and PFS and/or OS, measured in months from start of the study.

Assessment of risk of bias. The methodologic quality of all studies included in this review was assessed independently by two reviewers, using criteria adapted from Hayden et al (Hayden JA, et al: Ann Intern Med 144:427-437, 2006). For the first systematic review, the following four of the six original domains of potential bias were scored: (1) the study sample represents the population of interest; (2) loss to follow-up is not associated with key characteristics; (3) the prognostic factor is adequately measured in study participants; and (4) important potential confounders are accounted for. A low risk of bias was defined for these studies as four yeses and should be interpreted as a plausible bias unlikely to seriously alter the results. An unclear risk of bias was defined as three yeses and one response of no/unclear and should be interpreted as a plausible small bias that raises some doubt about the results. A high risk of bias was defined as $\leq$ two yeses and $\geq$ two responses of no/unclear and should be interpreted as a plausible bias that seriously weakens the evidence. In case of a response of no/unclear to the third domain (the prognostic factor is adequately measured in study participants), the study was always considered as having a high risk of bias, because there is no clear indication that the presence/induction of vitiligo was thoroughly assessed in studies not reporting any information on vitiligo. For the second systematic review, the following two additional domains were scored that involved the outcome parameter of survival, which could not be scored in the first systematic review: (5) the outcomes of interest (survival) are adequately measured in the study; and (6) the statistical analysis is appropriate for the design of the study. A low risk of bias was defined as a minimum of five yeses in response to these six questions. An uncertain risk of bias was defined as four yeses in response to the six questions. A high risk of bias was defined as $\geq$ three responses of no/unclear.