Cancer Immunol Immunother (2009) 58:145–151
DOI 10.1007/s00262-008-0506-5

SHORT COMMUNICATION

Vaccine-specific local T cell reactivity in immunotherapy-associated vitiligo in melanoma patients

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Received: 26 January 2008 / Accepted: 19 March 2008 / Published online: 5 April 2008

Abstract The occurrence of vitiligo in patients with melanoma is especially reported for patients undergoing immunotherapy. While vitiligo in these patients is thought to be related to an immune response directed against melanoma cells, solid evidence is lacking. Here we report local cytotoxic T cell reactivity in three melanoma patients who developed vitiligo, after experimental immunotherapy using dendritic cell vaccinations. Tetramer analysis showed that vaccine-induced T cells recognizing gp100 and tyrosinase are present at the vitiligo lesions. These T cells secrete IFN-γ and IL-2 upon peptide specific stimulation as well as upon recognition of the autologous tumor. We show that functional $CD8^+$ T cells specific for melanoma differentiation antigens used in a melanoma immunotherapy trial, do not only invade the tumor, but also the vitiligo lesions. This directly links vitiligo to the immuno-therapeutic intervention and supports the hypothesis that vitiligo is a marker of immunity against melanoma cells.

Keywords T cells · Melanoma · Vitiligo · Melanoma differentiation antigen

Abbreviations

CTL	Cytotoxic T lymphocyte
MDA	Melanoma differentiation antigen
DC	Dendritic cell
VIL	Vitiligo infiltrating lymphocyte
TIL	Tumor infiltrating lymphocyte

Introduction

Vitiligo is a common skin disease that is characterized by depigmented lesions associated with local destruction of melanocytes [12]. For melanoma patients the chance to develop vitiligo is estimated to be seven to tenfold higher compared to the general population [22], especially when they participate in an immunotherapeutic trial [25]. Based on numerous observations, development of de novo vitiligo lesions in melanoma patients is generally regarded as a favourable prognostic factor.

The etiology of vitiligo is not yet completely understood, but several lines of evidence suggest that both spontaneous and immunotherapy-associated vitiligo are immune-mediated processes. In spontaneous vitiligo, circulating anti-melanocytic

Electronic supplementary material The online version of this article (doi:10.1007/s00262-008-0506-5) contains supplementary material, which is available to authorized users.

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antibodies and lymphocyte infiltrations at the margins of progressive lesions are present [9, 17]. Furthermore, spontaneous vitiligo is correlated with circulating cytotoxic T lymphocytes (CTLs) specific for melanoma differentiation antigens (MDAs) [19]. Analysis of vitiligo infiltrating lymphocytes (VILs) isolated from vitiligo lesions showed a local enrichment of predominantly Melan-A specific T cells [11, 16, 30].

Numerous melanoma antigens are shared by normal melanocytes; amongst these are MDAs such as Melan-A/MART-1, gp100, tyrosinase and tyrosinase related proteins [3, 7]. In two murine melanoma models, it is shown that de novo development of vitiligo is associated with reduced tumor progression. In both models, MDA-specific CTLs play a crucial role in both tumor control and melanoma-associated vitiligo [13, 18]. Also in humans the appearance of vitiligo during the course of melanoma is thought to be caused by a cross-reactive immune response directed against melanoma cells, which explains the better prognosis in these patients reported in literature [4, 29].

Here we describe three melanoma patients who developed vitiligo after immunotherapeutic intervention. Patients were vaccinated with autologous monocyte-derived dendritic cells (DCs) loaded with gp100 and tyrosinase epitopes [5]. We demonstrate that CTLs against these epitopes can be detected in low percentages in the peripheral blood after vaccination. Interestingly, functional CTLs with the same specificity were observed in high percentages in both tumor and vitiligo lesions, which supports the hypothesis that the vitiligo is a direct cross-reactive effect of the anti-melanoma immunotherapy.

Material and methods

Patient selection

Melanoma patients with regional lymph node and distant metastases, participated in an experimental study using autologous monocyte-derived DCs loaded with HLA-A2.1 compatible tumor antigens gp100 and tyrosinase [5]. Only patients who developed vitiligo after immunotherapy, clinically confirmed by a dermatologist, were selected. Blood samples and 6 mm biopsies of perilesional vitiligo were obtained after informed consent. Approval from the local regulatory committee was obtained.

Preparation of vitiligo-infiltrating lymphocytes (VILs)

Six mm punch biopsies of the perilesional vitiligo-site (Fig. 1a) were obtained and cut in half. One part was cryopreserved for immunohistochemistry at a later timepoint,

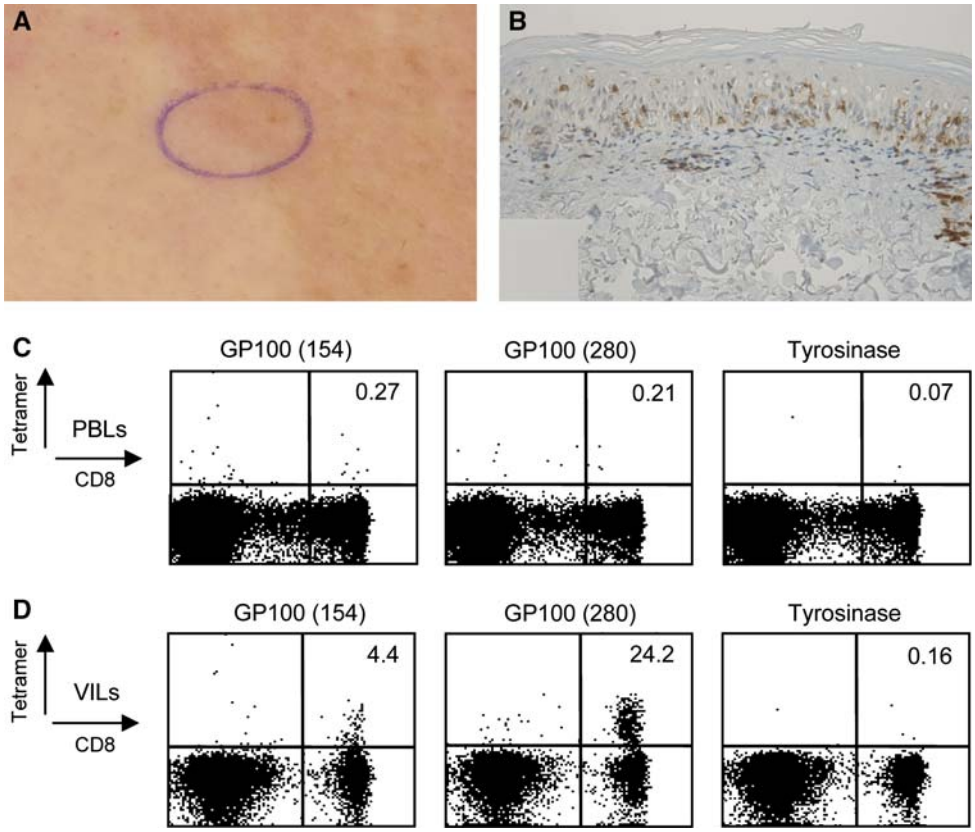

Fig. 1 Vaccine-specific CTLs accumulate at the vitiligo lesion. Perilesional 6 mm skin biopsies were taken from each patient (**a**). CD-3 staining of the periolesional skin showing lymphocyte infiltrates (**b**), original magnification ×200. Flowcytometry of peripheral blood lymphocytes (PBLs, **c**) and vitiligo infiltrating lymphocytes (VILs, **d**) from one representative patient (patient 3). From the scatterplot the lymphocytes were gated and double stained with anti-CD8-FITC and tetramer-PE. The numbers in the dot plots indicate the percentage of tetramer-reactive cells of the $CD8^+$ cell fraction

the other part was disrupted and a cell suspension was made by gentle squeezing in a sterile open filter chamber (NPBI, Amsterdam, The Netherlands) in RPMI/7% human serum (Sanquin, Nijmegen, The Netherlands) supplemented with IL-2 (100 U/ml). The cell suspension was placed in a 24-wells plate (Costar Badhoevedorp, The Netherlands). T cells were morphologically and functionally tested after 2 weeks of culture.

Preparation of tumor-infiltrating lymphocytes (TILs) and melanoma cell lines

TILs were obtained and cultured according to the same protocol as the vitiligo cell suspension (see above). A tumor cell line was generated by tumor culture in DMEM (Gibco—Invitrogen) supplemented with 10% fetal calf serum (Greiner). Fresh medium was added twice a week.

MHC Tetramer staining

VILs and TILs (50×10^3 cells in 10 µl) or 1×10^6 peripheral blood mononuclear cells in 10 µl, were incubated with directly labeled $gp100^{154–162}$, $gp100^{280–288}$, $tyrosinase^{369–376}$ and $Melan\text{-}A^{26–35}$ and $HIV^{77–85}$ tetrameric-MHC-HLA-A2.1 complexes for 60 min at room temperature. FITC-conjugated monoclonal antibodies directed against CD8 (Becton Dickinson) were added during the last 20 min. After washing, the samples were analyzed by flow cytometry. Percentage tetramer positive cells were calculated over the $CD8^+$ fraction. All samples were tested with $HIV^{77–85}$-HLA-A2.1-tetramers recognizing the irrelevant HIV-peptide SLYNTVATL as background control [5]. Control tetramer binding was less than 0.02% in all samples (supplementary figure 1).

Cytokine production

Production of cytokines by VILs and TILs were measured in response to specific stimuli (target:effector-ratio 1:1). Two different target cells were used: T2 cells pulsed with $gp100^{154–162}$, $gp100^{280–288}$, $tyrosinase^{369–376}$ or the irrelevant peptide $HIV^{77–85}$ and BLM cells (a melanoma cell line expressing HLA-A2.1 and no endogenous expression of gp100 and tyrosinase) transfected with control antigen G250, gp100 or tyrosinase. IFN-γ and IL-2 production were measured in supernatants after 16 hours by cytometric bead array according to the manufacturers protocol (Th1/Th2 Cytokine CBA 1; BD Pharmingen).

Antibodies and immunostaining

Regulatory T cell phenotype was assessed by flowcytometry using mAbs directly conjugated to fluorescent dyes. The FOXP3-FITC (clone PCH101, eBioscience, San Diago, CA, USA), CD4-APC, CD25-PE, CD127-PE-Cy5 (all Becton Dickinson) were used according to manufacturers protocols. Regulatory T cells were defined as $CD4^+FoxP3^+CD25^+CD127^-$.

For immunohistochemistry, the following mAbs were used: M2-7C10+M2-9E3 (Labvision, Fremont, CA, USA) against Melan-A, SP7 (Labvision) against CD3, HMB-45 (Dako, Glostrup, Denmark) against gp100 and T311 (Novocastra, Newcastle, UK) against tyrosinase [6].

Results and discussion

Patients

We prospectively followed three HLA-A2.1 positive patients with histologically documented melanoma who received DC-based immunotherapy and subsequently developed vitiligo (Table 1).

Patient 1. Patient 1 was diagnosed, two years prior to inclusion, with melanoma of the scalp with regional lymph node metastases (T3aN2aM0), for which a radical lymph node dissection was performed. She relapsed with regional lymph node and cutaneous metastases, which were resected and treated with adjuvant radiotherapy. At inclusion, this patient presented with multiple subcutaneous and lung metastases. The patient was vaccinated intranodally with antigen loaded mature DCs [5] but treatment was discontinued after 3 vaccinations because of progression of cutaneous metastases. She developed vitiligo in the flank during further tumor progression.

Patient 2. Patient 2 was diagnosed, twelve years prior to inclusion, with melanoma located on the right lower leg (T1aN0M0), which was surgically removed. From 1997 on, the patient relapsed with in transit and distant cutaneous metastases, for which he was treated with regional limb perfusion (melfalan) and dacarbazine. At inclusion, he was diagnosed with lymph node metastases and multiple (sub-)cutaneous metastases for which he was repeatedly vaccinated intranodally with antigen loaded mature DCs. After three vaccinations, the patient developed vitiligo in the neck region and dorsal side of both hands. To date, the vitiligo still progresses and affects the head, back, hands and legs.

Patient 3. Patient 3 was diagnosed, 6 months prior to inclusion, with a superficial spreading melanoma of the left thigh (T1a/bN0M0). At inclusion she presented with an inguinal lymph node metastasis, for which a radical lymph node dissection was performed. She was vaccinated intradermally in an adjuvant setting with antigen loaded mature DCs. After eight vaccinations, she developed a severe rash in the neck region, thorax, back and upper extremities and subsequent vitiligo in these areas.

Table 1 Patient characteristics and tetramer specific CTLs after immunotherapy

Patient/sex/age (years)	TNM (stage)	Localization	Prior treatment	Vaccinations[a]	TTP (months)	Current status (months)	Tetramer specific CTLs after immunotherapy[b]			
							gp100-154 epitope	gp100-280 epitope	Tyrosinase	Melan-A
1/F/20	M1b (IV)	Lung, skin, LN	Post-surgery RTx	3	7	AWD (21)				
						DTH	NA	NA	NA	NA
						Blood[c]	0.01	0.03	0.00	0.03
						Vitiligo	0.05	0.02	0.00	1.4
						Melanoma	0.32[d]	0.04	0.02	1.7[d]
2/M/71	M1a (IV)	Distant skin	Dacarbazine, Melfalan, DNCB cream	3	38	AWD (56)				
						DTH	0.06	0.04	0.15	NA
						Blood[c]	0.10	0.82	0.01	0.01
						Vitiligo	0.00	1.6	0.77	1.13
3/F/53	N1b (III)	LN	none	7	NDD	NDD (21)				
						DTH	0.01	0.04	0.01	NA
						Blood[c]	0.27	0.21	0.07	0.06
						Vitiligo	4.40	24.2	0.16	0.05

LN lymphnode, *TTP* time to progression, *AWD* alive with disease, *NDD* no detectable disease, *DTH* delayed type hypersensitivity-assay, *NA* not available

[a] Number of vaccinations prior to occurrence of vitiligo

[b] Percentage of tetramer specific CD8+ T cells compared to total CD8+ T cells

[c] No specific CTLs for gp100, tyrosinase and Melan-A were detected in the blood prior to immunotherapy

[d] Flowcytometry results for gp100 specific CTLs infiltrating the melanoma are shown in Fig. 3a and b

Vitiligo development in melanoma patients upon vaccination with DCs has been reported before [15, 24]. Although not investigated, it is currently believed that the vitiligo in these patients results from sensitization to antigens shared by melanocytes and melanoma cells. To address this question we took perilesional biopsies and studied the vitiligo infiltrating immune cells.

Vaccine specific lymphocytes infiltrate vitiligo lesions

Immunohistochemical analysis of the vitiligo in all three patients demonstrates perilesional infiltration of T cells (Fig. 1a, b). With tetramer analysis, $CD8^+$ T cells specific for one or more gp100 and tyrosinase HLA-A2.1 epitopes were detected at low frequencies in the peripheral blood after vaccination in all three patients. Gp100 or tyrosinase specific $CD8^+$ T cells were not detectable prior to vaccination (data not shown). Interestingly, specific CTLs were detected at increased concentrations in the vitiligo lesion compared to peripheral blood (Table 1; supplementary figure 1). This phenomenon was most obvious in patient 3 (Fig. 1c, d). The accumulation of these CTLs in the vitiligo lesions can result from specific migration [26] and/or a local proliferation advantage for MDA specific CTLs induced by perilesional antigen exposure.

Although the immunotherapy was not primarily directed against the Melan-A epitope, Melan-A specific CTLs were observed in the vitiligo lesions and tumor lesion of patient 1 and 2 (Table 1; Fig. 3). This finding is consistent with the observation that anti-tumor vaccines can have effects beyond their intrinsic specificity. It is shown that the interaction of vaccine specific T cells with melanoma cells may trigger a broad activation of other anti-tumor T cells, a phenomenon called epitope spreading [10, 14].

Vitiligo infiltrating lymphocytes produce IFN-γ after specific stimulation

To test whether vitiligo infiltrating lymphocytes (VILs) were functional, we stimulated these cells with gp100 or tyrosinase expressing target cells and measured subsequent IFN-γ and IL-2 production. VILs of patients 1 and 3 produce IFN-γ and IL-2 when exposed to gp100-target cells and VILs of patient 2 produce IFN-γ and IL-2 when exposed to both gp100 and tyrosinase-target cells. No cytokines were produced when VILs were exposed to unloaded control target cells (Fig. 2).

From patients 1 and 2 we were able to culture melanoma cell lines from metastases. Interestingly, the VILs also produced IFN-γ and IL-2 when stimulated by the autologous tumor cell line (Fig. 2). The cross reactivity of the VILs with autologous tumor cells further supports the hypothesis that the vitiligo is a marker of immunity against melanoma cells.

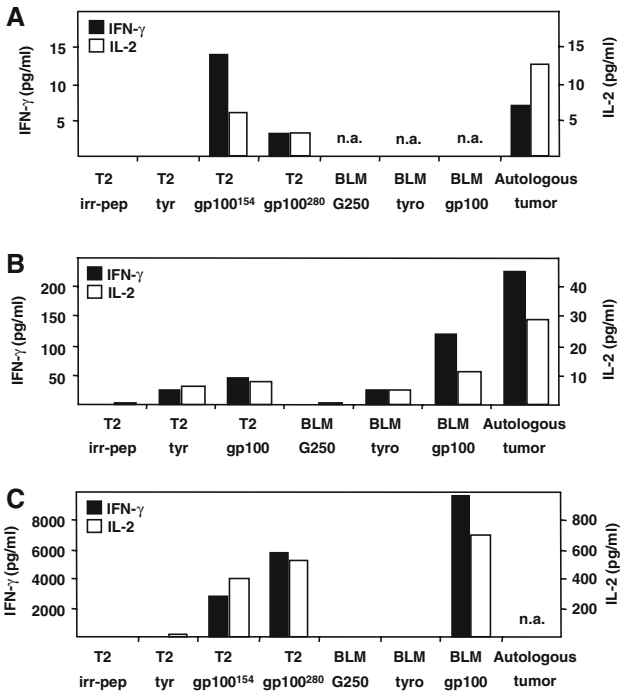

Fig. 2 Vitiligo infiltrating lymphocytes produce IFN-γ and IL-2 upon specific stimulation. IFN-γ (*black bars*) and IL-2 (*white bars*) production of VILs after stimulation with non-specific and specific stimuli of patient 1 (**a**) patient 2 (**b**) and patient 3 (**c**). T2 cells are loaded with respectively irrelevant peptide, tyrosinase peptide and gp100 peptides. BLM cells are transfected with respectively control protein G250, tyrosinase protein and gp100 protein (see also material en methods). From patients 1 and 2 we cultured melanoma cell lines, the VILs produced high levels of IFN-γ and IL-2 when stimulated by the autologous tumor cells. *NA* not available

Vitiligo lesions in our patients did not specifically occur at the vaccine injection-site, at the site of the primary tumor, or in close proximity of new metastases. From this we conclude that the melanoma-associated vitiligo is not caused by a direct and local side-effect of tumor destruction, but rather by the presence of circulating MDA-specific CTLs. Although we did not use MHC class II peptides in our vaccination protocol, our data do not exclude that specific $CD4^+$ cells are involved. MDA-specific epitopes recognized by $CD4^+$ T cells have been reported [31] and it is also known that melanocytes can present peptides in an MHC class II restricted manner [28]. These observations suggest that melanocyte destruction may not only depend on MHC class I-restricted cells.

Immunotherapy-associated vitiligo and prognosis

Our case series is too limited to afford decisive conclusions on the prognostic value of vitiligo on melanoma disease. The presence of durable non-progressive disease in patients 2 and 3 suggest that the immunotherapy induced vitiligo lesions are a favourable prognostic factor. We further show

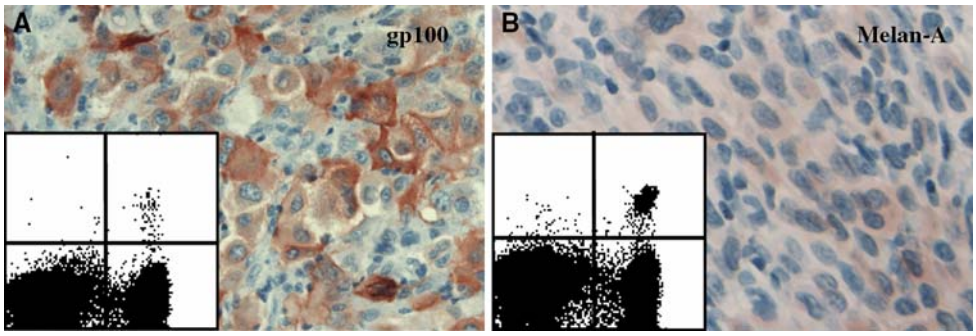

Fig. 3 Accumulation of MDA-specific CTLs in the melanoma metastasis of patient 1. Immunohistochemistry of parafin sections from the melanoma tumor of patient 1, showing melanoma cells that stain positive for the proteins gp100 (**a**) and Melan-A (**b**). Original magnification 200x. Small inserts show tetramer-analysis. $CD8^+$ T cells (horizontal axis) are plotted against $gp100^{154–162}$-epitope and Melan-$A^{26–35}$-epitope tetramers (*vertical axis*) showing 0.32% gp100 (**a**) and 1.7% Melan-A (**b**) specific CTLs (see also Table 1)

that all three patients have a relatively high number of MDA-specific T cells in the blood, DTH biopsies and melanoma. Earlier papers have described a correlation with the presence of MDA-specific T cells in these compartments and favourable outcome [2, 5].

Despite the presence of functional melanoma specific T cells, patient 1 had progressive disease. This is intriguing since the tumor expressed the MDAs gp100 and Melan-A (IHC, Fig. 3a, b) and was infiltrated by CTLs that specifically recognize these epitopes (inserts Fig. 3a, b; Table 1). Several mechanisms may account for the escape of melanoma cells to immune surveillance [21]. It has recently been published that $CD4^+FoxP3^+CD25^+$ regulatory T cells (Tregs) can infiltrate melanoma tumors and locally suppress cytotoxic anti-tumor responses, also in vaccinated melanoma patients [1]. Infiltration of melanoma tumors with Tregs correlates with poor prognosis [27]. In the melanoma metastasis of patient 1, we detected that 27% of the tumor infiltrating $CD4^+$ T cells consisted of $CD4^+FoxP3^+CD25^+CD127^-$ Tregs vs 4% of Tregs in the blood of this patient (data not shown).

Altogether, our data support an association between vitiligo and favourable outcome. However, active vaccine-induced vitiligo does not exclude tumor progression. The dichotomy between MDA-specific CTL responses in melanoma and vitiligo might result from quantitative CTL differences, the quality of the CTLs such as T cell receptor affinity and cytokine production [20], the amount of antigen presented [8, 23] and the different environmental conditions in which these T cells exert their function [21].

Concluding remarks

Melanoma-associated vitiligo is more often seen in patients who undergo immunotherapy. We demonstrate that immunotherapy against gp100 and tyrosinase antigens can induce specific and functional CTLs that invade both melanoma and vitiligo lesions. This directly links the occurrence of vitiligo to the immuno-therapeutic intervention.

Acknowledgments This study was supported by grants KUN 1999/1950, 2000/2301, 2003/2893, 2003/2917 and 2006/3699 from the Dutch Cancer Society and the TIL-foundation.

References

1. Appay V, Jandus C, Voelter V, Reynard S, Coupland SE, Rimoldi D, Lienard D, Guillaume P, Krieg AM, Cerottini JC, Romero P, Leyvraz S, Rufer N, Speiser DE (2006) New generation vaccine induces effective melanoma-specific CD8+ T cells in the circulation but not in the tumor site. J Immunol 177:1670
2. Banchereau J, Palucka AK, Dhodapkar M, Burkeholder S, Taquet N, Rolland A, Taquet S, Coquery S, Wittkowski KM, Bhardwaj N, Pineiro L, Steinman R, Fay J (2001) Immune and clinical responses in patients with metastatic melanoma to CD34(+) progenitor-derived dendritic cell vaccine. Cancer Res 61:6451
3. Boon T, Coulie PG, Van den Eynde B (1997) Tumor antigens recognized by T cells. Immunol Today 18:267
4. Das PK, van den Wijngaard RM, Wankowicz-Kalinska A, Le Poole IC (2001) A symbiotic concept of autoimmunity and tumour immunity: lessons from vitiligo. Trends Immunol 22:130
5. de Vries IJ, Bernsen MR, Lesterhuis WJ, Scharenborg NM, Strijk SP, Gerritsen MJ, Ruiter DJ, Figdor CG, Punt CJ, Adema GJ (2005) Immunomonitoring tumor-specific T cells in delayed-type hypersensitivity skin biopsies after dendritic cell vaccination correlates with clinical outcome. J Clin Oncol 23:5779
6. de Vries TJ, Smeets M, de Graaf R, Hou-Jensen K, Brocker EB, Renard N, Eggermont AM, van Muijen GN, Ruiter DJ (2001) Expression of gp100, MART-1, tyrosinase, and S100 in paraffin-embedded primary melanomas and locoregional, lymph node, and visceral metastases: implications for diagnosis and immunotherapy. A study conducted by the EORTC Melanoma Cooperative Group. J Pathol 193:13
7. Engelhard VH, Bullock TN, Colella TA, Sheasley SL, Mullins DW (2002) Antigens derived from melanocyte differentiation proteins: self-tolerance, autoimmunity, and use for cancer immunotherapy. Immunol Rev 188:136

8. Ferrone S, Marincola FM (1995) Loss of HLA class I antigens by melanoma cells: molecular mechanisms, functional significance and clinical relevance. Immunol Today 16:487
9. Garbelli S, Mantovani S, Palermo B, Giachino C (2005) Melanocyte-specific, cytotoxic T cell responses in vitiligo: the effective variant of melanoma immunity? Pigment Cell Res 18:234
10. Lally KM, Mocellin S, Ohnmacht GA, Nielsen MB, Bettinotti M, Panelli MC, Monsurro V, Marincola FM (2001) Unmasking cryptic epitopes after loss of immunodominant tumor antigen expression through epitope spreading. Int J Cancer 93:841
11. Lang KS, Caroli CC, Muhm A, Wernet D, Moris A, Schittek B, Knauss-Scherwitz E, Stevanovic S, Rammensee HG, Garbe C (2001) HLA-A2 restricted, melanocyte-specific CD8(+) T lymphocytes detected in vitiligo patients are related to disease activity and are predominantly directed against MelanA/MART1. J Invest Dermatol 116:891
12. Le Poole IC, van den Wijngaard RM, Westerhof W, Dutrieux RP, Das PK (1993) Presence or absence of melanocytes in vitiligo lesions: an immunohistochemical investigation. J Invest Dermatol 100:816
13. Lengagne R, Le Gal FA, Garcette M, Fiette L, Ave P, Kato M, Briand JP, Massot C, Nakashima I, Renia L, Guillet JG, Prevost-Blondel A (2004) Spontaneous vitiligo in an animal model for human melanoma: role of tumor-specific CD8+ T cells. Cancer Res 64:1496
14. Lurquin C, Lethe B, De Plaen E, Corbiere V, Theate I, van Baren N, Coulie PG, Boon T (2005) Contrasting frequencies of antitumor and anti-vaccine T cells in metastases of a melanoma patient vaccinated with a MAGE tumor antigen. J Exp Med 201:249
15. Mackensen A, Herbst B, Chen JL, Kohler G, Noppen C, Herr W, Spagnoli GC, Cerundolo V, Lindemann A (2000) Phase I study in melanoma patients of a vaccine with peptide-pulsed dendritic cells generated in vitro from CD34(+) hematopoietic progenitor cells. Int J Cancer 86:385
16. Mandelcorn-Monson RL, Shear NH, Yau E, Sambhara S, Barber BH, Spaner D, DeBenedette MA (2003) Cytotoxic T lymphocyte reactivity to gp100, MelanA/MART-1, and tyrosinase, in HLA-A2-positive vitiligo patients. J Invest Dermatol 121:550
17. Ongenae K, Van Geel N, Naeyaert JM (2003) Evidence for an autoimmune pathogenesis of vitiligo. Pigment Cell Res 16:90
18. Overwijk WW, Theoret MR, Finkelstein SE, Surman DR, de Jong LA, Vyth-Dreese FA, Dellemijn TA, Antony PA, Spiess PJ, Palmer DC, Heimann DM, Klebanoff CA, Yu Z, Hwang LN, Feigenbaum L, Kruisbeek AM, Rosenberg SA, Restifo NP (2003) Tumor regression and autoimmunity after reversal of a functionally tolerant state of self-reactive CD8+ T cells. J Exp Med 198:569
19. Palermo B, Campanelli R, Garbelli S, Mantovani S, Lantelme E, Brazzelli V, Ardigo M, Borroni G, Martinetti M, Badulli C, Necker A, Giachino C (2001) Specific cytotoxic T lymphocyte responses against Melan-A/MART1, tyrosinase and gp100 in vitiligo by the use of major histocompatibility complex/peptide tetramers: the role of cellular immunity in the etiopathogenesis of vitiligo. J Invest Dermatol 117:326
20. Palermo B, Garbelli S, Mantovani S, Scoccia E, Da Prada GA, Bernabei P, Avanzini MA, Brazzelli V, Borroni G, Giachino C (2005) Qualitative difference between the cytotoxic T lymphocyte responses to melanocyte antigens in melanoma and vitiligo. Eur J Immunol 35:3153
21. Phan GQ, Wang E, Marincola FM (2001) T-cell-directed cancer vaccines: mechanisms of immune escape and immune tolerance. Expert Opin Biol Ther 1:511
22. Schallreuter KU, Levenig C, Berger J (1991) Vitiligo and cutaneous melanoma. A case study. Dermatologica 183:239
23. Slingluff CL Jr., Colella TA, Thompson L, Graham DD, Skipper JC, Caldwell J, Brinckerhoff L, Kittlesen DJ, Deacon DH, Oei C, Harthun NL, Huczko EL, Hunt DF, Darrow TL, Engelhard VH (2000) Melanomas with concordant loss of multiple melanocytic differentiation proteins: immune escape that may be overcome by targeting unique or undefined antigens. Cancer Immunol Immunother 48:661
24. Tsao H, Millman P, Linette GP, Hodi FS, Sober AJ, Goldberg MA, Haluska FG (2002) Hypopigmentation associated with an adenovirus-mediated gp100/MART-1-transduced dendritic cell vaccine for metastatic melanoma. Arch Dermatol 138:799
25. Uchi H, Stan R, Turk MJ, Engelhorn ME, Rizzuto GA, Goldberg SM, Wolchok JD, Houghton AN (2006) Unraveling the complex relationship between cancer immunity and autoimmunity: lessons from melanoma and vitiligo. Adv Immunol 90:215
26. van den Wijngaard R, Wankowicz-Kalinska A, Le Poole C, Tigges B, Westerhof W, Das P (2000) Local immune response in skin of generalized vitiligo patients. Destruction of melanocytes is associated with the prominent presence of CLA+ T cells at the perilesional site. Lab Invest 80:1299
27. Viguier M, Lemaitre F, Verola O, Cho MS, Gorochov G, Dubertret L, Bachelez H, Kourilsky P, Ferradini L (2004) Foxp3 expressing CD4+CD25(high) regulatory T cells are overrepresented in human metastatic melanoma lymph nodes and inhibit the function of infiltrating T cells. J Immunol 173:1444
28. Wang S, Bartido S, Yang G, Qin J, Moroi Y, Panageas KS, Lewis JJ, Houghton AN (1999) A role for a melanosome transport signal in accessing the MHC class II presentation pathway and in eliciting CD4+ T cell responses. J Immunol 163:5820
29. Wankowicz-Kalinska A, Le Poole C, van den Wijngaard R, Storkus WJ, Das PK (2003) Melanocyte-specific immune response in melanoma and vitiligo: two faces of the same coin? Pigment Cell Res 16:254
30. Wankowicz-Kalinska A, van den Wijngaard RM, Tigges BJ, Westerhof W, Ogg GS, Cerundolo V, Storkus WJ, Das PK (2003) Immunopolarization of CD4+ and CD8+ T cells to Type-1-like is associated with melanocyte loss in human vitiligo. Lab Invest 83:683
31. Zarour HM, Kirkwood JM, Kierstead LS, Herr W, Brusic V, Slingluff CL Jr., Sidney J, Sette A, Storkus WJ (2000) Melan-A/MART-1(51–73) represents an immunogenic HLA-DR4-restricted epitope recognized by melanoma-reactive CD4(+) T cells. Proc Natl Acad Sci USA 97:400