

Melanoma antigens and their recognition by T cells

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Abstract. During the last 10 years many melanoma antigens recognized by T cells have been molecularly characterized. This review summarizes the main features of these antigens, including both classes I and II HLA-restricted peptides, and describes their classification into diverse groups according to the tissue distribution of the antigens. The different *in vitro* and *in vivo* immunogenicity of such antigens is then discussed leading to the conclusion that Melan-A/MART-1 is the strongest among those tested being frequently recognized by patients' T cells both *in vitro* and *in vivo*. However, no correlation was found between T-cell response of melanoma patients to Melan-A/MART-1 and clinical response when it was used for vaccination. Data are also presented that suggest, through an *ex vivo* analysis carried out with tetramers staining of melanoma-specific T cells, that only in a limited number of advanced patients does a specific immune response develop. This response, however, appears unable to effectively counteract metastatic melanoma growth. (Keio J Med 50 (2): 86–90, June 2001)

Key words: melanoma, antigens, T cells

The molecular characterization of human tumor antigens has opened a new era in cancer immunology by allowing a more rapid translation of information gained in basic research into clinical trials of immunotherapy.¹ Melanoma is the tumor that was more frequently studied thanks to several clinical reports suggesting its antigenicity and to the availability of tumor cell lines obtainable from melanoma patients and that could be extensively used for *in vitro* immunological studies.² Melanoma antigens have been studied for many years at cellular level thanks to the ability to derive T cell clones from peripheral blood lymphocytes (PBL) or lymph node lymphocytes of melanoma patients.³

Only molecular techniques, however, allowed cloning of genes encoding such protein antigens through the screening of cDNA expression tumor libraries by cytotoxic T lymphocytes (CTL) clones.⁴ An alternative approach to the identification of tumor (melanoma) antigens was used which is also dependent on the availability of patient's T cell clones but rests on a biochemical approach. This involves extraction of antigenic peptides from major histocompatibility complex (MHC)/peptide complexes expressed on the cell surface of tumor cells, their fractionation by re-

verse phase high performance liquid chromatography (HPLC), screening by anti-melanoma specific T cells and use of mass spectrometry to sequence the peptide of interest.⁵

By using either of these approaches many new melanoma antigens and their epitopes recognized by T cells in the context of class I HLA have been described.^{1,6} More recently, techniques became available that can be used also to molecularly characterize class II HLA-restricted melanoma epitopes recognized by CD4 T helper lymphocytes, a finding which paves the way to possibly more effective vaccines for the immunotherapy of melanoma patients. For a full list of these antigens see Renkvist, *et al.*⁷

Classification of Melanoma Antigens Recognized by T Cells

According to their molecular nature and tissue distribution, melanoma antigens can be classified as follows (Table 1):

Antigens predominantly expressed by tumor cells (or cancer/testis antigens)

Table 1 Classification of Human Melanoma Antigens Recognized by T Cells

Antigen	HLA-restriction	Example	Tissue distribution
Predominantly expressed by tumor cells (cancer/testis)	Classes I and II*	MAGE	Melanoma and other tumors; testis; placenta
Differentiation or melanocyte lineage-related	Classes I and II*	gp100	Normal and neoplastic melanocytes
Broadly expressed	Class I	p15	Normal and neoplastic tissues
Unique antigens	Classes I and II	CDK4	Melanoma cells of a single tumor only
Melanoma-specific antigens	Class I	TRP2-INT2	Shared among melanomas

* The protein antigen contains different epitopes restricted either by class I or class II HLA. MAGE: melanoma antigen, CDK4: cycline-dependent kinase 4, TRP2: tyrosinase-related protein-2.

This group includes antigens represented by normal proteins expressed by melanoma and other histologically different tumors but not by normal tissues with the exception of testis. The prototype of this group of antigens is MAGE-1 (melanoma antigen-1), the first human tumor antigen recognized by HLA-class I-restricted T cells which has been molecularly characterized.⁴ In addition to the well known MAGE, BAGE and GAGE families of antigens, recently a new cancer/testis antigen has been described and called NY-ESO-1 owing to the place where it was first found (New York) and the histologically origin of the tumor (esophageous cancer) from which the antigen was isolated by using patient's serum antibodies and the Serex technique.⁸

The *in vitro* and *in vivo* immunogenicity of these antigens is, in general, rather weak⁹ and no tumor-infiltrating lymphocytes (TIL) could ever be found in melanoma patients directed against these families of antigens. Generation of anti-MAGE-3 immunity both *in vitro* and *in vivo* was, however, recently obtained by using dendritic cells for its presentation to the immune system.¹⁰

Differentiation antigens recognized by class I HLA-restricted T cells

These antigens are shared between melanomas and normal melanocytes and are thus lineage-related normal proteins mostly involved in the biosynthesis of melanin.¹¹ These antigens are recognized by PBL, which can be stimulated *in vitro* to develop antigen-specific CTL.¹² Moreover, TIL recognizing such antigens *in vitro* in the presence of IL-2, were shown to cause clinical responses when re-infused *in vivo* in patients whose tumor cells express the cognate antigen.^{13,14} Therefore, melanocyte differentiation antigens appear to be immunogenic at least in a fraction of patients with a hierarchy that indicates Melan-A/MART1 as the most immunogenic one (see below).

Broadly expressed antigens

Other class I HLA-restricted antigens have been reported consisting of normal proteins expressed widely both on neoplastic and normal tissues, like p15 or oncoproteins (e.g. Her2/neu) or oncosuppressive molecules (p53, p16) (see Table 1). Interestingly, recent studies indicate that the protein domain of telomerase can also provide epitopes expressed mostly by cancer cells, including melanoma, and which are recognized by class I HLA-restricted T cells.¹⁵ It is possible that many normal protein epitopes expressed by normal tissues are below the threshold level of T-cell recognition, while their overexpression in tumor cells can trigger an anti-cancer response even by breaking a previously established tolerance. This may be the case for the antigen PRAME which is detectable by reverse transcribed PCR in melanoma and normal tissues but is not recognized by T cells in the latter thus becoming operationally tumor-specific.¹⁶

Melanoma-specific, class I HLA-restricted unique and shared antigens

Important antigens are those expressed exclusively by melanoma but not normal tissues. This group includes two subgroups of antigens. Those generated by point mutation of normal proteins and expressed by one single tumor only (unique antigens) like cycline-dependent kinase 4 (CDK4), β -catenin (see Table 2) that represent a biologically important feature of melanoma but, up to now, of impossible use in vaccination trials owing to the difficulty to prepare peptide-based vaccines at the single patient level. The other subgroup encompasses at least two antigens which are shared among melanomas without being found in normal tissues, including melanocytes. This kind of antigen, if immunogenic enough, will represent the ideal vaccine that should allow immunization of many patients without generating any toxic effect while sparing the normal tissues from any harmful immunological reaction. We found that HLA-A2 and HLA-A3-restricted epitopes are contained in these antigens whose molecular nature was not completely elucidated.^{17,18} However, at

Table 2 Melanoma-Specific Unique and Shared Antigens Recognized by T Cells

Antigen	HLA-restriction	Mechanism of generation
Unique		
CDK4	Class I	Point mutation
β -catenin	Class I	Point mutation
Mum-1	Class I	Point mutation
Mum-2	Class I	Point mutation
CDC27	Class II	Point mutation
LDLR/FUT	Class II	Chromosome rearrangement
Shared		
TRP2/INT	Class I	Translation of intronic sequences
GnT-V	Class I	Translation of intronic sequences

CDK4: cyclin-dependent kinase 4, TRP2: tyrosinase-related protein-2, GnT-V: acetylglucosaminyl transferase V.

least two members of this subgroup of tumor-specific antigens have been molecularly characterized (N-acetylglucosaminyl transferase V (GnT-V) and (TRP (tyrosinase-related protein) -2-INT2)); it is of note that in both cases the immunogenic epitopes were created by the translation of retained intronic sequences.^{19,20}

Class II HLA-restricted melanoma antigens

Immunogenicity of HLA class I-restricted melanoma antigens has been found to be weak and only occasionally do patients develop a strong CTL reactivity after immunization with molecularly defined vaccines. Among the many possible reasons of such a phenomenon, the lack of activation of T helper cells may be crucial inasmuch as CD4 T cells appear to be instrumental in eliciting a strong and sustained immune response against tumor antigens.²¹ Therefore, it is important to understand whether melanoma antigens include epitopes that can activate a Th1 driven immune reaction, which may ultimately result in melanoma cell destruction. In the last few years several class II HLA-restricted epitopes have been described. They fall into the group of differentiation antigens (e.g. tyrosinase, Melan-A/MART1)^{22,23} and cancer/testis antigens (e.g. MAGE-3, NY-ESO-1)²⁴⁻²⁶ (see Table 1) but, interesting enough, several additional epitopes were found to result from point mutations or chromosomal rearrangement of a variety of normal genes (see Table 3).^{27,28} For a complete list and features of class II HLA-restricted antigens see Renkvist, *et al.*⁷

It should be pointed out that, though the molecular identification of many melanoma antigens represents a crucial progress in tumor immunology, from a practical point of view the preparation of vaccines from a single patient neoplasm (which should contain the whole spectrum of potential antigens of that particular tumor) still remains an unsolved problem. It is likely that truly

Table 3 *In Vitro* Immunogenicity of Melanoma Antigens

Antigen	N. of positive reactions	%	HLA-restriction
	N. of patients tested		
Tyrosinase	8/24	33	A2, B44
gp100	5/26	19	A2, A3, Cw8
Melan-A/MART1	25/43	58	A2
MAGE-3	4/23	18	A1, A2
MAGE-1	2/7		A1, A24
GnT-V	3/9		A2

Immunogenicity was evaluated by induction of antigen-specific CTLs from PBL of melanoma patients after 1-5 weeks of stimulation with melanoma cells or peptide-pulsed APC. GnT-V: acetylglucosaminyl transferase V.

tumor-specific, immunogenic antigens are difficult to find and may include unique antigens that cannot be characterized in a time compatible with their use in metastatic melanoma patients in whom the tumor is progressing. However, the use of heat-shock proteins (HSP) which, at least in the mouse system, are known to bind the full repertoire of immunogenic tumor peptides, including the unique ones, and serve as chaperones for presentation of such peptides by dendritic cells may allow the need of antigen identification to be bypassed.²⁹ In fact, we have recently shown that HSP of 70 Kd purified from human melanomas efficiently present known melanoma antigens to patients' T lymphocytes.³⁰ This approach is feasible at individual patient level provided enough tumor tissue is available to purify HSP.

Recognition by T Cells *in vitro* and *in vivo*

Most of the antigens discussed above are recognized by patient's T cells *in vitro* after repeated stimulations and with a variable frequency in a population of melanoma patients. Results obtained by this approach, however, do not allow distinguishing between patients whose immune system was already primed *in vivo* against melanoma antigens owing to natural tumor growth, from patients whose T cells were primed *in vitro* by repeated antigenic stimulations. Nevertheless, the results of these experiments did demonstrate that patients often have T-cell precursors that can potentially recognize melanoma antigens. The question arises, however, which of the many known peptide epitopes expressed by melanoma cells were the most immunogenic ones and, therefore, the best candidates for a vaccination therapy.

Based on published data from several groups of investigators, one can establish a hierarchy in terms of *in vitro* immunogenicity of melanoma antigens used in the form of peptides pulsed onto antigen-presenting cells to stimulate patients' T cells (Table 3). From such

an analysis, it appears that Melan-A/MART-1_(27–35) is the peptide epitope more frequently recognized by patients' PBL after *in vitro* stimulation,³¹ possibly thanks to a higher frequency of T cell precursors against this antigen already present in normal donors³² as compared to other melanoma antigens. This conclusion was corroborated by subsequent studies of *ex vivo* enumeration of melanoma antigen-specific T lymphocytes with tetramers,³³ of immunization of HLA-A2-transgenic mice given different HLA-A2-restricted human melanoma peptides³⁴ and, finally, of vaccination trials showing that melanoma patients developed anti-Melan-A/MART-1 CTL responses with a frequency higher than that obtained by immunizing with gp100 or tyrosinase peptides.³⁵ It should be noted, however, that CTL response elicited in patients after vaccination with melanoma antigen peptides did not correlate with clinical responses.^{35–37}

The availability of tetramers and the possibility to study the *in vivo* distribution of tetramers plus T cells and their function *in vitro* without the need to culture these T cells, is shedding light on the complex interactions between melanoma growth, antigen expression, immune reactions and clinical outcome. In fact, Anichini and co-workers have shown that 40% of untreated metastatic melanoma patients possess Melan-A/MART-1-specific CD45RO⁺ memory T cells which display tumor cytotoxicity after two weeks of culture in the presence of IL-2 while being ineffective within metastatic lesions *in vivo* despite the presence of the HLA-A2 restricting molecule and of the MART-1 as shown by immunohistochemistry.³⁸ Moreover, a recent re-evaluation of melanoma patients by using MART-1 tetramers to select melanoma antigen-specific T cells has shown that the memory effector T cell phenotype (CD45RO⁺, CCR7[–]) can be found only in 25% of metastatic melanoma patients, appears late in the progression of the disease, and is associated with a poor prognosis.³⁹ From these studies it can be concluded that melanoma growth *in vivo* may activate a specific T-cell response against Melan-A/MART-1 but only after a large tumor burden has been reached, this response being then unable to efficiently control tumor growth.

This conclusion, however, is based on a few studies and on the evaluation of the T-cell response against a single melanoma antigen, namely Melan-A/MART-1, which may not necessarily reflect the general behaviour of the kinetics of the immune response to the whole, unknown spectrum of melanoma antigens.

Concluding Remarks

Many melanoma antigens recognized by T cells in the context of classes I or II MHC are now available and several of these antigens are being used as vac-

cines, singly or in combination (polyepitopes vaccines). However, we have still to find out how to make melanoma antigens more immunogenic to patients' immune system and to define the kinetics of the T cell response to such antigens during vaccination. The new technology of tetramers now allows such an analysis to be carried out at least for some antigens. A better knowledge of the interplay between melanoma cell antigens and the host's immune system may provide new insight to optimize future protocols of vaccination.

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References

1. Kawakami Y, Rosenberg SA: T-cell recognition of self peptides as tumor rejection antigens. *Immunol Res* 1996; 15: 179–190
2. Parmiani G, Anichini A, Fossati G: Cellular immune response against autologous human malignant melanoma: are *in vitro* studies providing a framework for a more effective immunotherapy? *J Natl Cancer Inst* 1990; 82: 361–370
3. Anichini A, Fossati G, Parmiani G: Clonal analysis of the cytolytic T-cell response to human tumors. *Immunol Today* 1987; 8: 385–389
4. van der Bruggen P, Traversari C, Chomez P, Lurquin C, De Plaen E, Van den Eynde B, Knuth A, Boon T: A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. *Science* 1991; 254: 1643–1647
5. Cox AL, Skipper J, Chen Y, Henderson RA, Darrow TL, Shabanowitz J, Engelhard VH, Hunt DF, Slingluff CL Jr: Identification of a peptide recognized by five melanoma-specific human cytotoxic T-cell lines. *Science* 1994; 264: 716–719
6. Castelli C, Rivoltini L, Andreola G, Carrabba M, Renkvist N, Parmiani G: T-cell recognition of melanoma-associated antigens. *J Cell Physiol* 2000; 182: 323–331
7. Renkvist N, Castelli C, Robbins PF, Parmiani G: A list of human tumor antigens recognized by T cells. *Cancer Immunol Immunother* 2001; 50: 3–15
8. Chen YT, Scanlan MJ, Sahin U, Türeci Ö, Gure AO, Tsang S, Williamson B, Stockert E, Pfreundschuh M, Old LJ: A testicular antigen aberrantly expressed in human cancers detected by autologous antibody screening. *Proc Natl Acad Sci USA* 1997; 94: 1914–1918
9. Chaux P, Vantomme V, Coulie P, Boon T, van der Bruggen P: Estimation of the frequencies of anti-MAGE-3 cytolytic T-lymphocyte precursors in blood from individuals without cancer. *Int J Cancer* 1998; 77: 538–542
10. Schuler-Thurner B, Dieckmann D, Keikavoussi P, Bender A, Maczek C, Jonuleit H, Roder C, Haendle I, Leisgang W, Dunbar R, *et al.*: Mage-3 and influenza-matrix peptide-specific cytotoxic T cells are inducible in terminal stage HLA-A2.1+ melanoma patients by mature monocyte-derived dendritic cells. *J Immunol* 2000; 165: 3492–3496
11. Anichini A, Maccalli C, Mortarini R, Salvi S, Mazzocchi A, Squarcina P, Herlyn M, Parmiani G: Melanoma cells and normal melanocytes share antigens recognized by HLA-A2-restricted cytotoxic T cell clones from melanoma patients. *J Exp Med* 1993; 177: 989–998

12. Rivoltini L, Kawakami Y, Sakaguchi K, Southwood S, Sette A, Robbins PF, Marincola FM, Salgaller ML, Yannelli JR, Appella E, *et al.*: Induction of tumor-reactive CTL from peripheral blood and tumor-infiltrating lymphocytes of melanoma patients by *in vitro* stimulation with an immunodominant peptide of the human melanoma antigen MART-1. *J Immunol* 1995; 154: 2257–2265
13. Robbins PF, el-Gamil M, Kawakami Y, Stevens E, Yannelli JR, Rosenberg SA: Recognition of tyrosinase by tumor-infiltrating lymphocytes from a patient responding to immunotherapy. *Cancer Res* 1994; 54: 3124–3126
14. Kawakami Y, Eliyahu S, Jennings C, Sakaguchi K, Kang X, Southwood S, Robbins PF, Sette A, Appella E, Rosenberg SA: Recognition of multiple epitopes in the human melanoma antigen gp100 by tumor-infiltrating T lymphocytes associated with *in vivo* tumor regression. *J Immunol* 1995; 154: 3961–3968
15. Vonderheide RH, Hahn WC, Schultze JL, Nadler LM: The telomerase catalytic subunit is a widely expressed tumor-associated antigen recognized by cytotoxic T lymphocytes. *Immunity* 1999; 10: 673–679
16. Ikeda H, Lethè B, Lehmann F, Van Baren N, Baurain JF, de Smet C, Chambost H, Vitale M, Moretta A, Boon T, Coulie PG: Characterization of an antigen that is recognized on a melanoma showing partial HLA loss by CTL expressing an NK inhibitory receptor. *Immunity* 1997; 6: 199–208
17. Anichini A, Mortarini R, Maccalli C, Squarcina P, Fleischhauer K, Mascheroni L, Parmiani G: Cytotoxic T cells directed to tumor antigens not expressed on normal melanocytes dominate HLA-A2.1-restricted immune repertoire to melanoma. *J Immunol* 1996; 156: 208–217
18. Mazzocchi A, Storkus WJ, Traversari C, Tarsini P, Maeurer MJ, Rivoltini L, Vegetti C, Belli F, Anichini A, Parmiani G, Castelli C: Multiple melanoma-associated epitopes recognized by HLA-A3-restricted CTLs and shared by melanomas but not melanocytes. *J Immunol* 1996; 157: 3030–3038
19. Guilloux Y, Lucas S, Brichard VG, Van Pel A, Viret C, De Plaen E, Brasseur F, Lethè B, Jotereau F, Boon T: A peptide recognized by human cytolytic T lymphocytes on HLA-A2 melanomas is encoded by an intron sequence of the N-acetylglucosaminyltransferase V gene. *J Exp Med* 1996; 183: 1173–1183
20. Lupetti R, Pisarra P, Verrecchia A, Farina C, Nicolini G, Anichini A, Bordignon C, Sensi M, Parmiani G, Traversari C: Translation of a retained intron in tyrosinase-related protein (TRP) 2 mRNA generates a new cytotoxic T lymphocyte (CTL)-defined and shared human melanoma antigen not expressed in normal cells of the melanocytic lineage. *J Exp Med* 1998; 188: 1005–1016
21. Toes RE, Ossendorp F, Offringa R, Melief CJ: CD4 T cells and their role in antitumor immune responses. *J Exp Med* 1999; 189: 753–756
22. Topalian SL, Rivoltini L, Mancini M, Markus NR, Robbins PF, Kawakami Y, Rosenberg SA: Human CD4+ T cells specifically recognize a shared melanoma-associated antigen encoded by the tyrosinase gene. *Proc Natl Acad Sci USA* 1994; 91: 9461–9465
23. Zarour HM, Kirkwood JM, Kierstead LS, Herr W, Brusica V, Slingluff CL Jr, Sidney J, Sette A, Storkus WJ: 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* 2000; 97: 400–405
24. Chauv P, Vantomme V, Stroobant V, Thielemans K, Corthals J, Luiten R, Eggermont AM, Boon T, van der Bruggen P: Identification of MAGE-3 epitopes presented by HLA-DR molecules to CD4(+) T lymphocytes. *J Exp Med* 1999; 189: 767–778
25. Manici S, Sturniolo T, Imro MA, Hammer J, Sinigaglia F, Noppen C, Spagnoli G, Mazzi B, Bellone M, Dellabona P, *et al.*: Melanoma cells present a MAGE-3 epitope to CD4(+) cytotoxic T cells in association with histocompatibility leukocyte antigen DR11. *J Exp Med* 1999; 189: 871–876
26. Zeng G, Touloukian CE, Wang X, Restifo NP, Rosenberg SA, Wang RF: Identification of CD4+ T cell epitopes from NY-ESO-1 presented by HLA-DR molecules. *J Immunol* 2000; 165: 1153–1159
27. Pieper R, Christian RE, Gonzales MI, Nishimura MI, Gupta G, Settlege RE, Shabanowitz J, Rosenberg SA, Hunt DF, Topalian SL: Biochemical identification of a mutated human melanoma antigen recognized by CD4(+) T cells. *J Exp Med* 1999; 189: 757–766
28. Wang RF, Wang X, Atwood AC, Topalian SL, Rosenberg SA: Cloning genes encoding MHC class II-restricted antigens: mutated CDC27 as a tumor antigen. *Science* 1999; 284: 1351–1354
29. Srivastava PK, Menoret A, Basu S, Binder RJ, McQuade KL: Heat shock proteins come of age: Primitive functions acquire new roles in an adaptive world. *Immunity* 1998; 8: 657–665
30. Castelli C, Ciupitu AM, Rini F, Rivoltini L, Mazzocchi A, Kiesling R, Parmiani G: Human heat shock protein 70 peptide complexes specifically activate antimelanoma T cells. *Cancer Res* 2001; 61: 222–227
31. Parmiani G: Future perspectives in specific immunotherapy of melanoma. *Eur J Cancer* 1998; 34(Suppl 3): S42–S47
32. Marincola FM, Rivoltini L, Salgaller ML, Player M, Rosenberg SA: Differential anti-MART-1/Melan A CTL activity in peripheral blood of HLA-A2 melanoma patients in comparison to healthy donors: evidence of *in vivo* priming by tumor cells. *J Immunother Emphasis Tumor Immunol* 1996; 19: 266–277
33. Romero P, Dunbar PR, Valmori D, Pittet M, Ogg GS, Rimoldi D, Chen JL, Liénard D, Cerottini JC, Cerundolo V: Ex vivo staining of metastatic lymph nodes by class I major histocompatibility complex tetramers reveals high numbers of antigen-experienced tumor-specific cytolytic T lymphocytes. *J Exp Med* 1998; 188: 1641–1650
34. Mateo L, Gardner J, Chen Q, Schmidt C, Down M, Elliott SL, Pye SJ, Firat H, Lemonnier FA, Cebon J, Suhrbier A: An HLA-A2 polyepitope vaccine for melanoma immunotherapy. *J Immunol* 1999; 163: 4058–4063
35. Jäger E, Bernhard H, Romero P, Ringhoffer M, Arand M, Karbach J, Ilsemann C, Hagedorn M, Knuth A: Generation of cytotoxic T-cell responses with synthetic melanoma-associated peptides *in vivo*: implications for tumor vaccines with melanoma-associated antigens. *Int J Cancer* 1996; 66: 162–169
36. Cormier JN, Salgaller ML, Prevette T, Barracchini KC, Rivoltini L, Restifo NP, Rosenberg SA, Marincola FM: Enhancement of cellular immunity in melanoma patients immunized with a peptide from MART-1 Melan-A. *Cancer J Sci Am* 1997; 3: 37–44
37. Marchand M, van Baren N, Weynants P, Brichard V, Dréno B, Tessier MH, Rankin E, Parmiani G, Arienti F, Humblet Y, *et al.*: Tumor regressions observed in patients with metastatic melanoma treated with an antigenic peptide encoded by gene MAGE-3 and presented by HLA-A1. *Int J Cancer* 1999; 80: 219–230
38. Anichini A, Molla A, Mortarini R, Tragni G, Bersani I, Di Nicola M, Gianni AM, Pilotti S, Dunbar R, Cerundolo V, Parmiani G: An expanded peripheral T cell population to a cytotoxic T lymphocyte (CTL)-defined, melanocyte-specific antigen in metastatic melanoma patients impacts on generation of peptide-specific CTLs but does not overcome tumor escape from immune surveillance in metastatic lesions. *J Exp Med* 1999; 190: 651–667
39. Dunbar PR, Smith CL, Chao D, Salio M, Shepherd D, Mirza F, Lipp M, Lanzavecchia A, Sallusto F, Evans A, *et al.*: A shift in the phenotype of Melan-A-specific CTL identifies melanoma patients with an active tumor-specific immune response. *J Immunol* 2000; 165: 6644–6652