

## Expression of gene *MAGE-A4* in Reed-Sternberg cells

Hervé Chambost, Nicolas Van Baren, Francis Brasseur, Danièle Godelaine, Luc Xerri, Sylvaine Just Landi, Ivan Theate, Joel Plumas, Guilio C. Spagnoli, Gerard Michel, Pierre G. Coulie, and Daniel Olive

Genes of the *MAGE-A* family are expressed in several types of solid tumors but are silent in normal tissues with the exception of male germline cells, which do not carry HLA molecules. Therefore, peptides encoded by *MAGE-A* genes are strictly tumor-specific antigens that can be recognized by CTL and constitute promising targets for immunotherapy. The expression of 6 genes of the *MAGE-A* family was tested with reverse transcrip-

tase-polymerase chain reaction in lymphoma samples. Among 38 samples of non-Hodgkin lymphoma, 1 anaplastic large cell lymphoma expressed genes *MAGE-A1*, *-A2*, *-A3*, *-A4*, and *-A12*, and 1 lymphoepithelioid T-cell lymphoma expressed gene *MAGE-A4*. Five of 18 samples (28%) from patients with Hodgkin disease expressed gene *MAGE-A4*. In tissue sections, staining by a monoclonal antibody that recognizes the *MAGE-A4*

protein was observed in 11 of 53 samples (21%) from patients with Hodgkin disease. In the positive samples, the Reed-Sternberg cells were strongly stained whereas the surrounding cells were not. These results indicate that Hodgkin disease may be a target for specific immunotherapy involving *MAGE-A4* antigens. (Blood. 2000;95:3530-3533)

© 2000 by The American Society of Hematology

### Introduction

Intensive chemotherapy and bone marrow transplantation have improved the disease-free survival of patients with non-Hodgkin lymphoma (NHL) and Hodgkin disease (HD). However, the relapse rate remains important, particularly in NHL. Clinical experience with allogeneic bone marrow transplantation demonstrated that immune-mediated graft-versus-lymphoma reactions may be induced in NHL patients, a situation that may be analogous to the graft-versus-leukemia (GVL) effect that participates in the cure of leukemia patients.<sup>1-6</sup> Immunotherapy involving the administration of immunostimulatory cytokines or donor lymphocytes seemed to improve the clinical outcome of a few lymphoma patients.<sup>7,8</sup> However, optimizing the antilymphoma effect of allogeneic reactions is clearly difficult, considering the mortality and morbidity associated with graft-versus-host disease. New immunotherapeutic tools would be necessary, at least as adjuvant treatment, to control residual disease.

Tumor-specific antigens consisting of peptides presented to autologous cytolytic T lymphocytes by class I human leukocyte antigens (HLAs) may constitute targets for specific immunotherapy.<sup>9</sup> Several types of antigens present on leukemia, lymphoma, or melanoma cells have been described: chimeric proteins resulting from gene translocations (for example, PML-RAR $\alpha$  in acute promyelocytic leukemia and p210 [BCR-ABL] in chronic myelocytic leukemia); proteinase 3, which is present in leukemia

cells but also in normal promyelocytes; the mucin MUC-1 in myeloma cells; the PRAME protein that appears to be overexpressed in many acute leukemias; or viral proteins such as LMP1 and LMP2 in lymphomas associated with Epstein-Barr virus infection.<sup>10-16</sup>

An interesting category of tumor antigens is encoded by genes such as *MAGE*, *BAGE*, *GAGE*, or *SSX*, which are expressed in many types of solid tumors but are silent in normal tissues with the exception of male germinal cells.<sup>9,17-19</sup> Because these cells do not carry HLA molecules, they cannot present antigenic peptides. Antigens encoded by such "cancer/testis" genes are therefore strictly tumor-specific and can be used safely in immunotherapy trials. Regressions of metastatic melanoma lesions were reported after immunization with an antigenic peptide encoded by gene *MAGE-A3* and presented by HLA-A1 molecules.<sup>20</sup>

*MAGE-A* is 1 of the 4 known *MAGE* gene families, and it comprises 12 genes. We reported that gene *MAGE-A1* was silent in bone marrow or blood samples from 48 leukemia patients.<sup>21</sup> The only hematologic malignancies in which *MAGE* gene expression has been detected are adult T-cell acute lymphoblastic leukemia and multiple myeloma.<sup>22-24</sup> Among the members of the *SSX* genes family, *SSX-2* was shown to be expressed in 4 of 11 NHL samples. Here we report on the analysis of *MAGE-A* gene expression in lymph node biopsies from lymphoma patients.

From INSERM U119 and Unité d'Immunologie des Tumeurs, Institut Paoli Calmettes, Marseilles, France; the Ludwig Institute for Cancer Research, Brussels, Belgium; Unité de Génétique Cellulaire, Université catholique de Louvain, Brussels, Belgium; Service d'Anatomopathologie, Institut Paoli Calmettes, Marseilles, France; Service d'Anatomopathologie, Cliniques Universitaires Saint-Luc, Brussels, Belgium; ETS Isère-Savoie and Research Group on Lymphoma, Unité UPRES 2021, Grenoble, France; the Department of Surgery, Research Division, University of Basel, Switzerland; and Service de Pédiatrie et d'Hématologie Pédiatrique, CHU Timone, Marseilles, France.

Supported in part by a Swiss National Fonds grant (no. 31-57'473.99) and by grants from the Association contre le Cancer, Brussels, Belgium; from CGER-Assurances and VIVA, Brussels, Belgium; from the Fonds National de la

Recherche Scientifique (TELEVIE grants), Brussels, Belgium; and from the Ligue Nationale contre le Cancer, France.

Submitted October 4, 1999; accepted January 30, 2000.

**Reprints:** Daniel Olive, INSERM U119 and Institut Paoli Calmettes, 27 Boulevard Leï Roure, Marseille, France; e-mail: olive@marseille.inserm.fr.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 U.S.C. section 1734.

© 2000 by The American Society of Hematology

## Patients, materials, and methods

### Control cell lines

Melanoma cell line MZ2-MEL-3.0 expresses genes *MAGE-A1*, *-A2*, *-A3*, and *-A6* but not *MAGE-A4*. Sarcoma cell line LB23-SARC expresses *MAGE-A4* but not *MAGE-A1*, *-A2*, *-A3*, *-A6*, or *-A12*. Melanoma cell line LB373-MEL expresses gene *MAGE-A12*. These cell lines were used as positive controls in the reverse transcriptase-polymerase chain reaction (RT-PCR) assays of *MAGE-A* genes expression.

### Normal lymphoid cells

Samples of peripheral blood mononuclear cells (PBMCs) (n = 8), phytohemagglutinin (PHA)-activated blood T cells (n = 3), normal bone marrow (n = 5), normal lymph nodes (n = 5), and cord blood cells (n = 4) from 25 patients without leukemia or lymphoma were used as controls in RT-PCR assays.

### Patients

Fifty-six lymphoma patients were included in the study: 38 with NHL and 18 with HD. Diagnosis was based on conventional morphologic examination of paraffin-embedded material, fixed with formalin or Bouin's fluid. The slides were examined by 2 pathologists (L.X. and I.T.) and, when required, diagnoses were refined with immunohistochemistry using monoclonal antibodies (MAbs) recognizing B cells, T cells or Reed-Sternberg cells. According to the REAL classification,<sup>25</sup> these lymphoid neoplasms were distributed as follows: 29 B-cell NHLs (2 small lymphocytic lymphomas, 5 mantle cell lymphomas, 14 follicle center lymphomas, 1 marginal zone B-cell lymphoma, 6 diffuse large B-cell lymphomas, and 1 plasmacytoma/plasma cell lymphoma), 9 T-cell NHLs (3 precursor T-lymphoblastic lymphomas, 5 anaplastic large cell lymphomas, and 1 lymphoepithelioid peripheral T-cell lymphoma), and 18 HD samples: 2 (HD1 and HD2) of type I (lymphocytic predominance), 8 (HD3-10) of type II (nodular sclerosis), 7 (HD11-17) of type III (mixed cellularity), and 1 (HD18) of type IV (lymphocyte depletion). Fifty-three HD samples, including 3 of type I, 34 of type II, 14 of type III, and 2 of type IV, were used for the immunohistochemical analysis. HD samples from 2 HD patients (HD3 and HD4) belonged to both series (RT-PCR analysis and immunohistochemistry).

### Lymph node biopsies

Lymph nodes were either frozen in liquid nitrogen immediately after surgical excision, or they were minced to obtain a suspension of cells that were frozen in RPMI (GIBCO BRL, Gaithersburg, MD) with 37.5% (vol/vol) fetal calf serum (GIBCO BRL) and 10% dimethyl sulfoxide and stored in liquid nitrogen. For patient HD3, cryopreserved lymph node cells were thawed, treated by trypsin ethylenediaminetetraacetic acid, and separated by positive immunoselection with CD30 MAb and goat anti-mouse magnetic beads to obtain fractions enriched or depleted in CD30<sup>+</sup> cells before RNA extraction.

### RT-PCR assay

Total cellular RNA was isolated by the guanidine-isothiocyanate/cesium chloride procedure<sup>26</sup> or with TRIzol (GIBCO BRL). Complementary DNA (cDNA) from 2 µg of total RNA was obtained by incubation at 42°C for 90 minutes with oligo(dT) primer and 200 units of Moloney murine leukemia virus RT (GIBCO BRL). PCR amplification was performed on 2.5% of the cDNA with 0.625 units of Taq DNA polymerase in a final volume of 25 µL. Reaction mixtures were heated at 94°C for 4 minutes and subjected to amplification for 30 (*MAGE-A1* to *-A6*) or 32 cycles (*MAGE-A12*) consisting of 1 minute at 94°C; 1 minute (2 minutes for *MAGE-A6* and *-A12*) at 72°C (*MAGE-A1* and *-A3*), 70°C (*MAGE-A6*), 68°C (*MAGE-A4*), 67°C (*MAGE-A2*), or 62°C (*MAGE-A12*); and 1 minute (2 minutes for *MAGE-A6* or 3 minutes for *MAGE-A12*) at 72°C. Final extension was obtained with 15 minutes at 72°C. The *MAGE*-specific primers were

described previously,<sup>18,27</sup> except for *MAGE-A12*: 5'-GCCCTCCACT-GATCTTTAGCAA-3' (exon 3)<sup>18</sup> and 5'-CGTTGGAGGTCAGAGAA-CAG-3' (exon 1). Each primer was chosen in a different exon to avoid false positives caused by DNA contamination of the RNA preparation. Samples with degraded RNA were excluded by testing the expression of the β-actin gene. The amounts of amplified products were evaluated visually on ethidium bromide-stained agarose gels and identified by comparing the size of the band with the molecular weight marker ΦX174 (GIBCO BRL). Bands of test samples were compared with those resulting from RT-PCR performed on serial dilutions (1:1, 1:3, 1:9, 1:27) of RNA from a positive control cell line: MZ2-MEL-3.0 for *MAGE-A1*, *-A2*, *-A3*, *-A6*; LB23-SARC for *MAGE-A4*; and LB373-MEL for *MAGE-A12*.

### Immunohistochemistry

Lymph node biopsy specimens from HD were formalin-fixed and embedded in paraffin. Immunohistochemical detection of the *MAGE-A4* protein was performed on paraffin sections previously heated for 10 minutes in the ChemMate Buffer for Antigen Retrieval (DAKO, Trappes, France) with the mouse MAb 57B.<sup>28</sup> The positive control was a sample of normal testis, whereas negative control consisted of replacing antibody 57B by an irrelevant isotype-matched MAb. The slides were processed using the alkaline phosphatase LSAB+ detection kit (DAKO) and the New Fuschin Substrate System (DAKO), according to the manufacturer's recommendations, and counterstained with hematoxylin.

## Results

### RT-PCR analysis of *MAGE-A* expression in lymph nodes from NHL patients

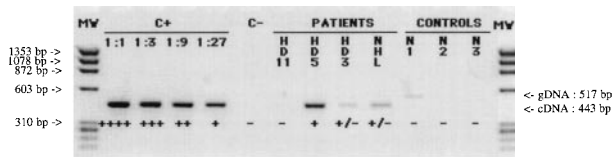
We tested 38 samples corresponding to various types of NHL. None of the 29 B-cell lymphomas expressed any of the 6 *MAGE-A* genes that were tested, whereas 2 of the 9 T-cell lymphomas were positive for at least 1 *MAGE-A* gene (Table 1). One anaplastic large-cell lymphoma expressed genes *MAGE-A1*, *-A2*, *-A3*, *-A4*, and *-A12* at a significant level, whereas expression of gene *MAGE-A6* was not detected. A low amount of *MAGE-A4* transcript

Table 1. Expression of *MAGE-A* genes

Patients	Pathology (according to the REAL classification)	Fraction of <i>MAGE</i> -positive samples
B-cell NHL (n = 29)	Small lymphocytic lymphoma	0/2
	Mantle cell lymphoma	0/5
	Follicle center lymphoma	0/14
	Marginal zone B-cell lymphoma	0/1
	Diffuse large B-cell lymphoma	0/6
	Plasmacytoma/plasma cell lymphoma	0/1
T-cell NHL (n = 9)	Total B-cell NHL	0/29
	Precursor T-lymphoblastic lymphoma	0/3
	Anaplastic large cell lymphomas	1/5*
	Lymphoepithelioid peripheral T-cell lymphoma	1/1*
	Total T-cell NHL	2/9 (22.2%)
HD (n = 18)	Type I (lymphocyte predominance)	0/2
	Type II (nodular sclerosis)	4/8*
	Type III (mixed cellularity)	1/7*
	Type IV (lymphocyte depletion)	0/1
	Total HD	5/18 (27.8%)

Expression of *MAGE-A* genes was determined semiquantitatively with RT-PCR as explained in the legend to Figure 1.

\*Levels of expression of the individual *MAGE-A* genes in the positive samples were as follows: 1 anaplastic large cell lymphoma: *MAGE-A1*+, *A2*+, *A3*+, *A4*+, and *A12*+; 1 lymphoepithelioid cell lymphoma: *MAGE-A4*±; 4 HD type II: 1 *MAGE-A4*+ (HD4), 1 *MAGE-A4*+ (HD5), and 2 *MAGE-A4*± (HD3, HD6); and 1 HD type III: *MAGE-A4*+ (HD17).



**Figure 1. RT-PCR amplification products of gene *MAGE-A4*.** Products were obtained with lymph node samples from patients with HD (HD11, HD5, HD3) or NHL and obtained with samples of normal lymphoid cells (N1, lymph node; N2, PBMC; N3, PHA-activated peripheral T cells). C+, positive control sarcoma line LB23-SARC. C-, negative control (no RNA present in the RT reaction). Amplification of *MAGE-A4* transcripts gives a band of 443 base pairs, distinct from that of the amplified genomic sequence of 517 base pairs (faintly visible in line N1). The levels of gene expression were evaluated by comparing band intensities with those obtained for serial dilutions (1:1, 1:3, 1:9, 1:27) from the positive and scored as follows: + + + +, 100%; + + +, 30% to 100%; + +, 10% to 30%; +, 3% to 10%; or  $\pm$ , less than 3%. Absence of RT-PCR product is indicated as -. RNA integrity was assessed by RT-PCR amplification of the  $\beta$ -actin mRNA (data not shown).

was detected in 1 sample of lymphoepithelioid peripheral T-cell lymphoma (Figure 1).

#### RT-PCR analysis of *MAGE-A* expression in lymph nodes from HD patients

Eighteen samples were tested. Gene *MAGE-A4* was found to be expressed in 5 of them, whereas the other *MAGE-A* genes were never detected (Table 1). The levels of *MAGE-A4* gene expression were assessed semiquantitatively as indicated in Table 1 and Figure 1. Among the 4 samples of HD type II that were tested, 1 (HD4) expressed gene *MAGE-A4* at a level that corresponded to 10% to 30% of that found in the reference sarcoma cell line, whereas the other 3 expressed 3% to 10% (HD5) or less than 3% of that level (HD3 and HD6). The positive sample of HD type III (HD17) expressed 3% to 10% of that level. Representative results are shown in Figure 1 for patient HD11 (-), HD5 (+), and HD3 ( $\pm$ ). Expression of genes *MAGE-A1*, *-A2*, *-A3*, *-A4*, *-A6*, and *-A12* was not detected in any of the 25 samples of normal hematopoietic cells (PBMCs, PHA-activated peripheral T cells, bone marrow, benign lymph node, cord blood cells) (Figure 1).

We used a monoclonal antibody against CD30/Ki-1, which is present on Reed-Sternberg cells, to sort CD30<sup>+</sup> and CD30<sup>-</sup> cells from a lymph node of patient HD3. *MAGE-A4* expression, analyzed with RT-PCR on the 2 cell populations, was restricted to the CD30<sup>+</sup> cells (data not shown).

#### Immunohistochemical detection of the *MAGE-A4* protein in HD lymph nodes

The low level of expression of *MAGE-A4* detected in 4 of 5 lymph nodes from HD patients could result either from a low level of transcription of the gene in most of the cells present in the sample

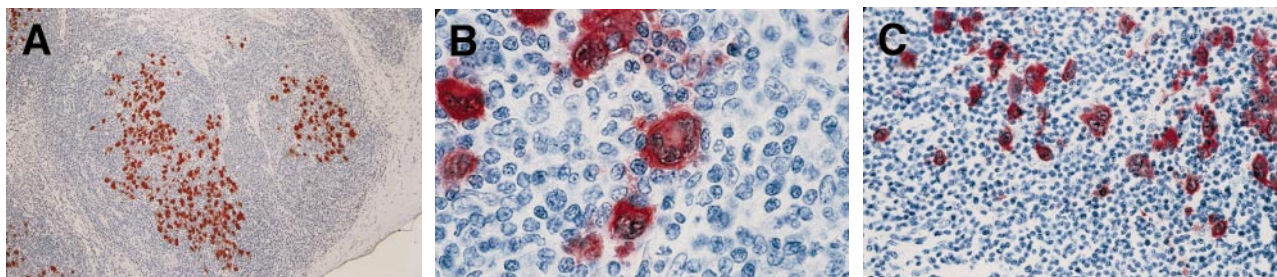
or from a higher expression by a minority of the cells. The latter possibility was obviously relevant, considering that Reed-Sternberg cells represent usually less than 5% to 10% of the total cell population in HD lymph nodes. We therefore analyzed paraffin sections of lymph nodes from 53 HD patients with MAb 57B. This antibody recognizes cells transfected with cDNAs encoding *MAGE-A1*, *-A2*, *-A3*, *-A4*, *-A6*, and *-A12* in tissue sections, and it stains the tumors that express gene *MAGE-A4* and only these tumors, regardless of the expression of the other *MAGE-A* genes.<sup>28</sup>

Antibody 57B stained 11 of 53 HD samples (21%), whereas no sample was stained by an isotype-matched control antibody. The positive samples included 1 of 3 HD type I, 8 of 34 HD type II, 2 of 14 HD type III, and 0 of 2 HD type IV. Representative results from patients HD3 and HD4 are shown in Figure 2. Positive cells are distributed in the different nodules (Figure 2A). At higher magnification, the staining is clearly observed in Reed-Sternberg cells (Figure 2B and 2C). Even though a weak background staining is present around some Reed-Sternberg cells, the reactive small lymphocytes and histiocytes are not stained.

## Discussion

We report the expression of *MAGE-A* genes in 7 of 56 lymph nodes from lymphoma patients. Wide differences were observed according to the type of pathology. Whereas B-cell lymphomas did not express any of the 6 *MAGE-A* genes that were tested, expression was found in 2 of 9 T-cell lymphomas. A higher proportion of positive samples has been reported previously in T-cell leukemias, which expressed gene *MAGE-A1*.<sup>23</sup> A notable difference between the 2 diseases is the pathogenesis of the T-cell leukemias, namely its frequent association with human T cell leukemia-lymphoma virus (HTLV-1) in Japan and the Philippines but not in European countries.

In 20% to 25% of lymph nodes from HD patients, we found expression of gene *MAGE-A4* but not of any of the other members of the *MAGE-A* gene family that were tested. The level of *MAGE-A4* expression was at least 10 times lower than that found in a control sarcoma cell line. These results matched with the observation that the *MAGE-A4* protein was detected only in Reed-Sternberg cells, which represent only 5% to 10% of the total cell population in most samples of HD lymph nodes. The proportion of samples that expressed gene *MAGE-A4* (28%), detected with RT-PCR, was similar to the proportion of samples in which a specific staining was observed with antibody 57B (21%). In addition, in the 2 positive samples that were analyzed with the 2 techniques, *MAGE-A4* expression was associated with the staining



**Figure 2. Labeling of Reed-Sternberg cells with anti-*MAGE-A4* MAb 57B in nodular sclerosing lymph nodes.** Patients include HD3 (A and B) and HD4 (C) at low (A), high (B) and intermediate (C) magnification. (B) and (C) correspond to *MAGE-A4* expression in HD3. Formalin-fixed sections were deparaffinized, heated in a citrate buffer, and incubated with MAb 57B. The antibody was detected with biotinylated secondary antibodies and avidin-coupled alkaline phosphatase. The chromogenic substrate was New Fuschin, and hematoxylin was used to counterstain the tissue.



of Reed-Sternberg cells by the anti-MAGE-A4 antibody. This MAGE-A4 positivity restricted to Reed-Sternberg cells validates the concept that HD patients whose lymph node samples display low levels of *MAGE-A4* gene expression (less than 3% of the level found in the reference cell line) should also be considered eligible for *MAGE-A4*-based immunotherapy. Our results are in line with the recent observation, based on a global gene expression analysis, that *MAGE-A4* is expressed in Reed-Sternberg cells.<sup>29</sup>

Interestingly, all 7 *MAGE-A*-positive lymphoma samples identified in this study expressed *MAGE-A4*, but only 1 of them expressed other *MAGE-A* genes in addition to *MAGE-A4*. All 5 *MAGE-A*-positive HD samples expressed only *MAGE-A4*. This observation contrasts with the frequent coexpression of several *MAGE-A* genes in solid tumors.<sup>18</sup> Unlike the other *MAGE-A* genes, *MAGE-A4* contains several alternative promoters.<sup>30</sup> It is therefore possible that 1 of them is specifically activated in some lymphoma cells, possibly through lymphoma-specific transcription factors.

Reed-Sternberg cells carry HLA class I molecules, have func-

tional TAP1 (transporter-associated protein) and TAP2 transporters, and bear the B7.1 and B7.2 costimulatory molecules.<sup>31-34</sup> Therefore, they may be targets of anti-MAGE-A4 T-cell responses. Reed-Sternberg cells from Epstein-Barr virus-positive patients were shown to be lysed by cytolytic T lymphocytes recognizing peptides encoded by the *LMP1* or *LMP2* genes of Epstein-Barr virus, indicating that these cells were indeed capable to process and present antigenic peptides derived from endogenous proteins.<sup>16,35</sup> It is therefore worth considering the possibility of vaccinating Hodgkin patients, selected on the basis of the expression of gene *MAGE-A4* by their tumor cells, with *MAGE-A4*-encoded antigens.

## Acknowledgments

We are particularly grateful to Mrs. M. Swinarska and M. Van Malderen for excellent technical assistance. We thank Dr R. Costello for helpful discussions.

## References

- Weiden PL, Flournoy N, Thomas ED, et al. Anti-leukemic effect of graft versus host disease in human recipients of allogeneic marrow grafts. *N Engl J Med*. 1979;300:1068.
- Apperley JF, Jones L, Hale G, Goldman JM. Bone marrow transplant for patients with chronic myeloid leukemia: T cell depletion with Campath-1 reduces the incidence of graft versus host disease but may increase the risk of leukemia relapse. *Bone Marrow Transplant*. 1988;1:53.
- Sullivan KM, Weiden PL, Storb R, et al. Influence of acute and chronic graft-versus-host disease on relapse and survival after bone marrow transplantation from HLA-identical siblings as treatment of acute and chronic leukemia. *Blood*. 1989;73:1720.
- Chopra R, Goldstone AH, Pearce R, et al. Autologous versus allogeneic bone marrow transplantation for non-Hodgkin's lymphoma: a case-controlled analysis of the European Bone Marrow Transplantation Group Registry Data. *J Clin Oncol*. 1992;10:1690.
- Philipps GL, Herzog RH, Lazarus HM, Fay JW, Griffith R, Herzog GP. High dose chemotherapy, fractionated total body irradiation and allogeneic transplantation for malignant lymphoma. *J Clin Oncol*. 1986;4:480.
- Jones RJ, Ambinder RF, Piantadosi S, Santos GW. Evidence of a graft-versus-lymphoma effect associated with allogeneic bone marrow transplantation. *Blood*. 1991;77:649.
- Nagler A, Ackerstein A, Or R, Naparstek E, Slavina S. Immunotherapy with recombinant human interleukin-2 and recombinant interferon- $\alpha$  in lymphoma patients postautologous marrow or stem cell transplantation. *Blood*. 1997;89:3951.
- Slavina S, Nagler A. Immunotherapy in conjunction with autologous and allogeneic blood or marrow transplantation in lymphoma. *Ann Oncol*. 1998;9: S31.
- Boon T. Human tumor antigens recognized by T lymphocytes. *J Exp Med*. 1996;183:725.
- Gambacorti-Passerini C, Grignani F, Arienti F, Pandolfi PP, Pelicci P, Parmiani G. Human CD4+ lymphocytes specifically recognize a peptide representing the fusion region of the hybrid protein PML-RAR $\alpha$  present in acute promyelocytic cells. *Blood*. 1993;81:1369.
- Bocchia M, Wentworth PA, Southwood S, et al. Specific binding of leukemia oncogene fusion protein peptides to HLA class I molecules. *Blood*. 1995;85:2680.
- Ten Bosch GJA, Toornvielt AC, Friede T, Melief CJM, Leeksa OC. Recognition of BCR-ABL positive leukemic blasts by human CD4+ T cells elicited by in vitro immunization with a BCR-ABL breakpoint peptide. *Blood*. 1996;88:3522.
- Nieda M, Nicol A, Kikuchi A, et al. Dendritic cells stimulate the expansion of bcr-abl specific CD8+ T cells with cytotoxic activity against leukemic cells from patients with chronic myelogenous leukemia. *Blood*. 1998;91:977.
- Barratt-Boyes SM. Making the most of mucin: a novel target for tumor immunotherapy. *Cancer Immunol Immunother*. 1996;43:142.
- Sing AP, Ambinder RF, Hong DJ, et al. Isolation of Epstein-Barr virus specific cytotoxic T lymphocytes that lyse Reed-Sternberg cells: implications for immune-mediated therapy for EBV+ Hodgkin's disease. *Blood*. 1997;89:1978.
- Van Baren N, Chambost H, Ferrant A, et al. PRAME, a gene encoding an antigen recognized on a human melanoma by cytolytic T cells, is expressed in acute leukaemia cells. *Br J Haematol*. 1998;102:1376.
- Van der Bruggen P, Traversari C, Chomez P, et al. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. *Science*. 1991;254:1643.
- De Plaen E, Arden K, Traversari C, et al. Structure, chromosomal localization and expression of 12 genes of the MAGE family. *Immunogenetics*. 1994;40:360.
- Türeci O, Chen YT, Sahin U, et al. Expression of SSX genes in human tumors. *Int J Cancer*. 1998;77:19.
- Marchand M, van Baren N, Weynants P, 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.
- Chambost H, Brasseur F, Coulie P, et al. A tumor-associated antigen expression in human haematological malignancies. *Br J Haematol*. 1993;84:524.
- Shichijo S, Tsunosue R, Masuoka K, et al. Expression of the MAGE gene family in human lymphocytic leukemia. *Cancer Immunol Immunother*. 1995;41:95.
- Shichijo S, Sagawa K, Brasseur F, Boon T, Itoh K. MAGE-1 gene is expressed in T-cell leukemia. *Int J Cancer*. 1996;65:709.
- van Baren N, Brasseur F, Godelaine D, et al. Genes encoding tumor-specific antigens are expressed in human myeloma cells. *Blood*. 1999;94:1156.
- Harris NL, Jaffe ES, Stein H, et al. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood*. 1994;84:1361.
- Davis LG, Dibrner MD, Battey JF. *Basic Methods in Molecular Biology*. New York, NY: Elsevier; 1986.
- De Smet C, Lurquin C, Van der Bruggen P, De Plaen E, Brasseur F, Boon T. Sequence and expression pattern of the human MAGE-2 gene. *Immunogenetics*. 1994;39:121.
- Landry C, Brasseur F, Spagnoli G, et al. Monoclonal antibody B57 stains tumor tissues that express gene MAGE-A4. *Int J Cancer*. In press.
- Cosman J, Annunziata CM, Barash S, et al. Reed-Sternberg cell genome expression supports a B-cell lineage. *Blood*. 1999;94:411.
- De Plaen E, Naerhuyzen B, De Smet C, Skikora JP, Boon T. Alternative promoters of gene MAGE-4a. *Genomics*. 1997;40:305.
- Murray P, Constandinou CM, Crocker J, Young L, Ambinder R. Analysis of major histocompatibility complex class I, TAP expression, and LMP2 sequence in Epstein-Barr virus-positive Hodgkin's disease. *Blood*. 1998;92:2477.
- Delabie J, Ceuppens JL, Vandenberghe P, de Boer M, Coorevits L, De Wolf-Peeters C. The B7/BB1 antigen is expressed by Reed Sternberg cells of Hodgkin's disease and contributes to the stimulating capacity of Hodgkin's disease-derived cell lines. *Blood*. 1993;82:2845.
- Van Gool SW, Delabie J, Vandenberghe P, Coorevits L, De Wolf-Peeters C, Ceuppens JL. Expression of B7-2 (CD86) molecules by Reed Sternberg cells of Hodgkin's disease. *Leukemia*. 1997;11:46.
- Xerri L, Devillard E, Hossain J, Olive D, Birg F. In vivo expression of the CTLA4 inhibitory receptor in malignant and reactive cells from human lymphoma. *J Pathol*. 1997;183:182.
- Lee SP, Constandinou CM, Thomas WA, et al. Antigen presenting phenotype of Hodgkin Reed Sternberg cells: analysis of the HLA class I processing pathway and the effects of interleukin-10 on Epstein-Barr virus-specific cytotoxic T-cell recognition. *Blood*. 1998;92:1020.