

NY-ESO-1 and LAGE-1 Cancer-Testis Antigens Are Potential Targets for Immunotherapy in Epithelial Ovarian Cancer¹

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ABSTRACT

Cancer-testis (CT) antigens are expressed in a variety of cancers, but not in normal adult tissues, except for germ cells of the testis, and hence appear to be ideal targets for immunotherapy. In an effort to examine the potential of NY-ESO-1 and LAGE-1 CT antigens for immunotherapy in epithelial ovarian cancer (EOC), we examined the expression of these antigens by reverse transcription-PCR (RT-PCR) and immunohistochemistry (IHC) in a large panel of EOC tissues and cell lines. Sera from a subgroup of the patients were tested for NY-ESO-1/LAGE-1 antibody by ELISA. The data indicated that four ovarian cancer cell lines were positive for one or both CT antigens. Expression of NY-ESO-1 in EOC was demonstrated by RT-PCR and/or IHC in 82 of 190 (43%) specimens. NY-ESO-1 expression by IHC ranged from homogeneous to heterogeneous pattern. LAGE-1 mRNA expression was present in 22 of 107 (21%) tumor tissues. Overall, the expression of either NY-ESO-1 or LAGE-1 mRNA was present in 42 of 107 (40%) EOC specimens and coexpression of both antigens was demonstrated in 11% of specimens. Antibody to NY-ESO-1/LAGE-1 was present in 11 of 37 (30%) patients whose tumors expressed either NY-ESO-1 or LAGE-1. Detectable antibodies were present for up to 3 years after initial diagnosis. Although there was no statistically significant relation between expression of NY-ESO-1/LAGE-1 antigen and survival, the data showed aberrant expression of NY-ESO-1 and LAGE-1 by IHC/RT-PCR in a significant proportion of EOC patients. These findings indicate that NY-ESO-1 and LAGE-1 are attractive targets for antigen-specific immunotherapy in EOC.

INTRODUCTION

Ovarian cancer is the leading cause of death from gynecologic malignancies. There are more than 23,000 cases annually in the United States, and 14,000 women can be expected to die from the disease each year (1). Despite modest improvements in response rates, progression-free, and median survival as a result of adjuvant platinum and paclitaxel chemotherapy after cytoreductive surgery, overall survival rates for patients with advanced EOC³ and ovarian-like malignancies (primary peritoneal) remain disappointing (2). This has been attributed to several reasons. First, in contrast to most other solid tumors, >75% of EOC patients present with advanced stage disease (FIGO III or IV). The 5-year survival rates are 51% for women diagnosed with regional disease and 25% for those diagnosed with distant disease. Second, although most patients initially respond to platinum and paclitaxel chemotherapy, including complete responses, the relapse rate is ~85%. Within 2 years of cytoreductive surgery and

systemic chemotherapy, tumors usually reoccur, and once relapse occurs, there is no known curative therapy. Thus, there is a need to develop additional therapeutic approaches for the management of this disease. A proposed strategy for minimizing the risk of recurrent disease is immunotherapy. Patients who demonstrate complete response to frontline surgery and chemotherapy could be considered for immunotherapy, with the presumption that the majority do in fact have micrometastases. However, the development of successful immunotherapeutic strategies requires the identification and characterization of immunogenic ovarian cancer antigens that will be recognized by the host immune system, leading to tumor rejection.

The development of approaches for analyzing humoral (3) and cellular (4) immune reactivity to cancer in the context of the autologous host has led to the molecular characterization of tumor antigens recognized by autologous CD8⁺ T cells (5) and/or antibodies (6). As a consequence of these advances, human tumor antigens defined to date can be classified into one or more of the following categories: (a) differentiation antigens, *e.g.*, tyrosinase (7), melan-A/MART-1 (8, 9), and gp100 (10); (b) mutational antigens, *e.g.*, CDK4 (11), β -catenin (12), caspase-8 (13), and P53 (14); (c) amplification antigens, *e.g.*, Her2/neu (15) and P53 (16); (d) splice variant antigens, *e.g.*, NY-CO-37/PDZ-45 (14) and ING1 (17); (e) viral antigens, *e.g.*, human papillomavirus (18) and EBV (19); and (f) CT antigens, *e.g.*, MAGE (20), NY-ESO-1 (21), and LAGE-1 (22). The CT antigens are a distinct and unique class of differentiation antigens. The defining characteristics of these antigens are the high levels of expression in adult male germ cells, but generally not in other normal adult tissues, and aberrant expression in a variable proportion of a wide range of different cancer types.

To be considered for antigen-specific immunotherapy of any tumor type, including EOC, an ideal candidate antigen should not only demonstrate high frequency expression in the tumor tissues and restricted expression in normal tissues, but also evidence for inherent immunogenicity. With regard to CT antigens, NY-ESO-1, initially defined by serological analysis of recombinant cDNA expression libraries in esophageal cancer (23), is particularly immunogenic, eliciting both cellular and humoral immune responses in a high proportion of patients with advanced NY-ESO-1-expressing tumors (24, 25). In contrast, cellular and humoral immune responses to other CT antigens appear to be less frequent. In the present study, we analyzed the expression pattern of NY-ESO-1 in a large number of EOC patients. We have also investigated the composite expression of LAGE-1, another CT antigen with 94% homology to NY-ESO-1 (22). In addition, we examined the evolution of serum antibody titers to NY-ESO-1 and LAGE-1 over extended periods of time in patients with EOC. Our results show that a significant proportion of human EOCs express NY-ESO-1 and/or LAGE-1 tumor antigens. In addition, we demonstrate the presence of humoral immune response in a group of EOC patients with NY-ESO-1- and LAGE-1-expressing tumors. These findings suggest that the NY-ESO-1 and LAGE-1 CT antigens are promising candidates for cancer-specific immunotherapy in EOC.

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³ The abbreviations used are: EOC, epithelial ovarian cancer; CT, cancer-testis; RT-PCR, reverse transcription-PCR; IHC, immunohistochemistry; mAb, monoclonal antibody; CI, confidence interval.

MATERIALS AND METHODS

Patients and Specimens. Flash-frozen tissue specimens were obtained from patients undergoing debulking surgery for EOC at the Roswell Park Cancer Institute (Buffalo, NY) between 1995 and 2002. An expanded set of formalin-fixed paraffin-embedded archival ovarian tumor specimens was also obtained from the institutional paraffin archive resource. All tissue specimens were collected under an approved protocol from the Institutional Review Board. All pathology specimens were reviewed in our institution, and tumors were classified according to WHO criteria (26). In a subset of the patients, serum samples were available over extended periods of time during the course of disease. The medical records of the patients were also retrospectively reviewed under an approved Institutional Review Board protocol. The review included out- and inpatient treatment, including surgery and chemotherapy. Study outcomes included overall survival and time to progression, each measured from the time of definitive surgery. Progression was defined as objective evidence of recurrence because all therapy was given in the adjuvant setting. The duration of overall survival was the interval between diagnosis and death. Observation time was the interval between diagnosis and last contact (death or last follow-up). Data were censored at the last follow-up for patients with no evidence of recurrence, progression, or death.

Cell Lines. Five ovarian cancer cell lines, SVOV3, OVCA-429, OVCA-432, SK-OV-3, and OVCAR-3, were purchased from the American Type Culture Collection and grown in the recommended media under standard conditions. The immortalized human normal ovarian surface epithelial cell lines, IOSE and HOSE, were gifts from Dr. Nancy Auersperg (University of British Columbia, Vancouver, BC) and Dr. Sam Mok (Harvard University, Boston, MA), respectively.

Total Tissue RNA Isolation. Total tissue RNA was isolated from frozen tumor tissues and from ovarian cancer cell lines by use of the TRIReagent (Molecular Research Center Inc, Cincinnati, OH) according to the manufacturer's protocol. Potentially contaminating DNA was removed by treating with RNase-free DNase I (Boehringer-Mannheim, Mannheim, Germany). After phenol treatment and drying, RNA was dissolved in RNase-free H₂O. The resulting RNA concentration was measured spectrophotometrically (GeneQuant; Amersham Pharmacia Biotech Ltd., Cambridge, United Kingdom), and the quality of the RNAs was checked by electrophoresis on 1% agarose gel.

RT-PCR Analysis of NY-ESO-1 and LAGE-1 Expression. Two micrograms of each RNA sample were subjected to cDNA synthesis using the Ready-To-Go first strand synthesis kit (Pharmacia, Uppsala, Sweden). PCR was subsequently performed to analyze expression of NY-ESO-1 and LAGE-1. The primers for NY-ESO-1 were ESO1A (5'-CACACAGGATC-CATGGATGCTGCAGATGCCG-3') and ESO1B (5'-CACACAAAGCTTG-GCTTAGCGCCTCTGCCCTG-3'), and the primers for LAGE-1 were LAGE1A (5'-CTGCGCAGGATGGAAGGTGCC-3') and LAGE-1B (5'-CACACAAAGCTTGGCTTAGCGCCTCTGCCCTG-3'). Amplification for both gene products was 1 min at 94°C, 1 min at 60°C, and 1.5 min at 72°C for 35 cycles. These cycles were followed by a 10-min elongation step at 72°C. Testicular tissue was used as a positive control. The PCR products were 341 and 338 bp for NY-ESO-1 and LAGE-1, respectively, and were visualized by ethidium bromide staining after separation over a 1.5% agarose gel.

IHC. Tumor specimens were fixed with buffered formalin and embedded in paraffin. Sections (5 µm) were placed on glass slides, heated at 60°C for 20 min, and then deparaffinized with xylene and ethanol. For antigen retrieval, tumor specimens mounted on glass slides were immersed in preheated antigen retrieval solution (DAKO high pH solution; DAKO, Carpinteria, CA) for 20 min and allowed to cool for 20 min at room temperature. After the inactivation of endogenous peroxidase, mAb to NY-ESO-1 (clone ES121) was then added at a concentration of 2.5 µg/ml and incubated overnight at 4°C. ES121 has been described previously (27). The primary antibody was detected with a biotinylated antimouse IgG (DAKO). Diaminobenzidine tetrahydrochloride was then added for development for 10 min, followed by counterstaining with hematoxylin solution.

The extent of immunohistochemical reactivity was graded as follows: negative, focal, staining of single cells or small clusters of cells (approximately <5% cells stained); +, 5–25% of cells stained; ++, >25–50% of cells stained; +++, >50–75% of cells stained; and +++++, >75% of cells stained. Negative control slides omitting the primary antibody were included in

all assays. At present, no mAb is available that distinguishes NY-ESO-1 and LAGE-1.

ELISA. Recombinant NY-ESO-1 and LAGE-1 truncated proteins (25) at a concentration of 1 µg/ml in coating buffer [15 mM Na₂CO₃, 30 mM NaHCO₃ (pH 9.6), 0.02% NaN₃] were adsorbed to 60 × 10 TC microwell plates (Nunc, Roskilde, Denmark) at 10 µl/well overnight at 4°C. Plates were washed with PBS and blocked overnight at 4°C with 10 µl/well of 2% BSA in PBS. After washing, 10 µl/well of serum dilutions in 2% BSA were added and incubated for 2 h at room temperature. Plates were washed, and 10 µl/well diluted secondary antibody-2% BSA were added (goat anti-human IgG-AP; Southern Biotechnology, Birmingham, AL) and incubated for 1 h at room temperature. Plates were washed, incubated with 10 µl/well of substrate solution (Attophase substrate; JBL Scientific, San Louis Obispo, CA) for 25 min at room temperature, and immediately read (Cyto-Fluor 2350; Millipore, Bedford, MA). Sera were tested over a range of 4-fold dilutions from 1:100 to 1:100,000, as described previously (25).

Statistical Analysis. All statistical analyses were performed with the SPSS software (28). Statistical correlations were calculated using Pearson's *r*. The distributions of NY-ESO-1 and LAGE-1 expression and clinical outcome were analyzed by the χ^2 test. Estimated survival distributions were calculated by the method of Kaplan and Meier (29), and tests of significance with respect to survival distributions were based on the log-rank test (30). No adjustments were made for multiple comparisons.

RESULTS

Study Population. A total of 107 flash-frozen EOC tissues were investigated by RT-PCR, and an expanded panel of 143 archival EOC specimens was analyzed by IHC. Thus, flash-frozen tissues only were available for analysis in some cases, whereas in other cases only paraffin sections were available. For 62 cases, frozen and archival specimens were available. The total number of tissue specimens examined by RT-PCR and/or IHC was 190. The characteristics of the study population are presented in Table 1. The median age of the study population was 61 years (range, 22–89 years), and the median duration of follow-up was 25 months (range, 1–126 months). As expected, the majority of patients presented with grade 3 tumors (88%), at stage IIIc (78%) and with serous histology (79%). A complete response to therapy was achieved in 97 of the 190 patients (51%), and a partial response was achieved in 81 patients (42%), whereas the remaining patients had no response. The median estimated overall survival for all patients was 55 months (CI, 38–71 months), whereas the median disease-free survival, excluding patients with persistent/progressive disease after initial therapy, was 33 months (CI, 23–42). The 5-year disease-free and overall survivals for the entire study population were 31% (CI, 18%–44%) and 46% (CI, 36%–56%), respectively.

Expression of NY-ESO-1 and LAGE-1 mRNA in EOC. Expression of NY-ESO-1 and LAGE-1 mRNA in epithelial ovarian tumor specimens was investigated by RT-PCR (Fig. 1). The intensities of the PCR products were heterogeneous, and some specimens yielded only faint amplicon bands. These were scored positive only if the result could be reproduced by a repeated RNA extraction and specific RT-PCR from the same tumor specimen. Cases with very low transcript levels that were not reproducibly positive were not regarded as positive. The normal ovarian surface epithelial cell line, IOSE, expressed LAGE-1, whereas the HOSE cell line did not express either tumor antigen. Four of the five ovarian cancer cell lines tested (80%) were positive for one or both tumor antigens. The SK-OV-3 cell line demonstrated dual expression of NY-ESO-1 and LAGE-1, whereas SVOV-3 was negative for both antigens (Fig. 1; Table 2). NY-ESO-1 mRNA expression was detected in 32 of 107 (30%) and LAGE-1 mRNA expression in 22 of 107 (21%) tumors. Coexpression of NY-ESO-1 and LAGE-1 mRNA was demonstrated in 12 of 107 (11%) specimens. Overall, expression of either NY-ESO-1 or

Table 1 Patient characteristics

Characteristics	
Evaluable patients	190
Age [median (range)], years	61 (22–89)
Follow-up ^a [median (range)], months	25 (0.7–126)
FIGO stage, n (%)	
IA	5 (2%)
IB	1 (1%)
IC	4 (2%)
IIB	4 (2%)
IIC	7 (4%)
IIIA	1 (1%)
IIIB	4 (2%)
IIIC	149 (78%)
IV	15 (8%)
Histology, n (%)	
Papillary serous	143 (79%)
Clear cell	8 (4%)
Endometrioid	8 (4%)
Mucinous	4 (2%)
Undifferentiated	6 (4%)
Others (transitional, mixed, carcinosarcoma)	13 (7%)
Grade, n (%)	
1	11 (6%)
2	10 (5%)
3	169 (88%)
Response to frontline therapy, n (%)	
Complete response	97 (51%)
Partial response	81 (42%)
Progression	3 (2%)
Unknown	9 (5%)
Recurrences, ^b n (%)	
Recurrence/persistent disease	138 (78%)
Current status, n (%)	
Alive, NED ^c	53 (28%)
Alive with disease	59 (31%)
Dead of disease	74 (39%)
Dead from other causes	3 (2%)
CT antigen status (RT-PCR and/or IHC), n (%)	
NY-ESO-1 positive	82/190 (43%)
LAGE-1 positive (RT-PCR only)	22/107 (21%)
NY-ESO-1 and/or LAGE-1 positive	42/107 (40%)
Humoral immune response, n (%)	
NY-ESO-1	10/37 (30%)
LAGE-1	3/6 (50%)

^a Median survival for all patients: 55 (CI, 37.6–71.41) months.

^b Median disease-free survival (excluding patients with persistent disease): 33 (CI, 23–42) months.

^c NED, no evidence of disease.

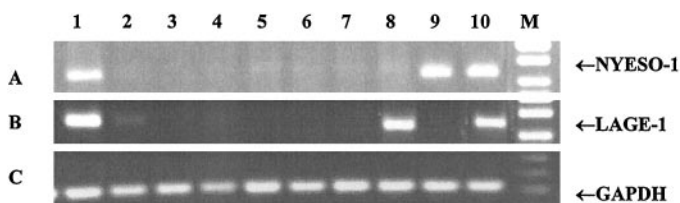


Fig. 1. RT-PCR analysis for NY-ESO-1 and LAGE-1 mRNA expression yielded 341- and 338-bp products, respectively. Shown is mRNA from testis (Lane 1), IOSE (Lane 2), HOSE (Lane 3), SVOV3 (Lane 4), OVCAR-3 (Lane 5), OVCA-429 (Lane 6), OVCA-432 (Lane 7), SK-OV-3 (Lane 8), and two EOC specimens (Lanes 9 and 10). GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

LAGE-1 mRNA was observed in 42 of 107 (40%) of EOC specimens (Table 3).

Immunohistostaining of NY-ESO-1. NY-ESO-1 exhibited intense immunostaining in testis (Fig. 2a), and the staining was restricted to germ cells. No reactivity in other structures of the testis was noted. The germ cells revealed nuclear and cytoplasmic staining that was typically strongest in the early cells of germ cell maturation. Positive staining was observed in 62 of 143 (43%) archival, formalin-fixed, paraffin-embedded ovarian cancer samples. Reactivity was mostly heterogeneous, *i.e.*, present in discrete areas of the tumors (focal or “+” staining according to our grading). Panels b–f in Fig. 2 show examples of focal through homogeneous staining patterns. The

predominant expression pattern was heterogeneous (focal, +, ++), occurring in 41 of 62 (66%) of NY-ESO-1-positive specimens, whereas the remaining 21 of 62 (34%) demonstrated +++ or ++++ staining (Table 4).

Considering all 190 tissue samples analyzed by RT-PCR and/or IHC in this study, positivity for NY-ESO-1 by either method was demonstrated in 82 (43%) of the tumors (Table 5). In the subset of 62 tumor specimens cotype for NY-ESO-1 by RT-PCR and IHC, there was a significant correlation between RT-PCR and IHC ($r = 0.03$). In 12 specimens, ES121 immunostaining was present in the absence of demonstrable mRNA expression by RT-PCR. All of these specimens revealed a restricted ES121 immunoreactivity (focal to ++ according to our IHC grading), some consisting of only single positive cells. No discrepancy between IHC and RT-PCR was observed in all of the tumor tissues with homogeneous (+++ or ++++) immunoreactivity. In nine tumor tissues, NY-ESO mRNA was present in the absence of ES121 immunoreactivity. These cases consistently showed weak bands by RT-PCR.

Correlation of NY-ESO-1 and LAGE-1 Expression with Clinical Outcome. The analysis of CT antigen expression and clinicopathological characteristics are presented in Tables 3, 4, and 5. Patients whose tumors expressed NY-ESO-1 by IHC and/or RT-PCR (Table 5)

Table 2 RT-PCR results for mRNA expression in five ovarian cancer cell lines and two normal ovarian surface epithelial cell lines

Antigens	Cell lines						
	SVOV3	OVCA-429	OVCA-432	SK-OV-3	OVCAR-3	IOSE	HOSE
NY-ESO-1	–	+	+	+	+	–	–
LAGE-1	–	–	–	+	–	+	–

Table 3 Correlation between NY-ESO-1 and LAGE-1 mRNA expression and clinicopathological features in EOC

Pathological and clinical features	NY-ESO-1 positive	LAGE-1 positive
All tumors ^a (n = 107)	32 (30%)	22 (21%)
Age [median (range)], years	61.0 (22–84)	63.0 (42–89)
Follow-up [median (range)], months	29.2 (2.1–109)	22.7 (1–83)
Tumor grade (n)		
G1		
G2	4	2
G3	28	20
FIGO stage (n)		
Ia		2
Ib		
Ic	1	
IIb		1
IIC		1
IIIA	1	
IIIB		1
IIIC	27	12
IV	3	5
Histology (n)		
Papillary serous	24	14
Clear cell	1	3
Endometrioid		
Mucinous	2	1
Undifferentiated		1
Others	5	3
Clinical response (n)		
Complete response	14	8
Partial response	16	11
Progression		1
Unknown	2	2
Total recurrences/persistent disease (n)	24	16
Dead of disease ^b (n)	12	9
Alive with disease (n)	13	8
Alive with no evidence of disease (n)	7	5

^a The expression of either NY-ESO-1 or LAGE-1 mRNA was found in 42/107 (40%) of EOC specimens.

^b Median survival for patients with NY-ESO-1 mRNA-positive and -negative tumors was 50 (CI, 19–81) and 57 (CI, 43–71) months, respectively ($P = 0.72$). Median survival for patients with LAGE-1 mRNA-positive and -negative tumors could not be determined ($P = 0.22$).

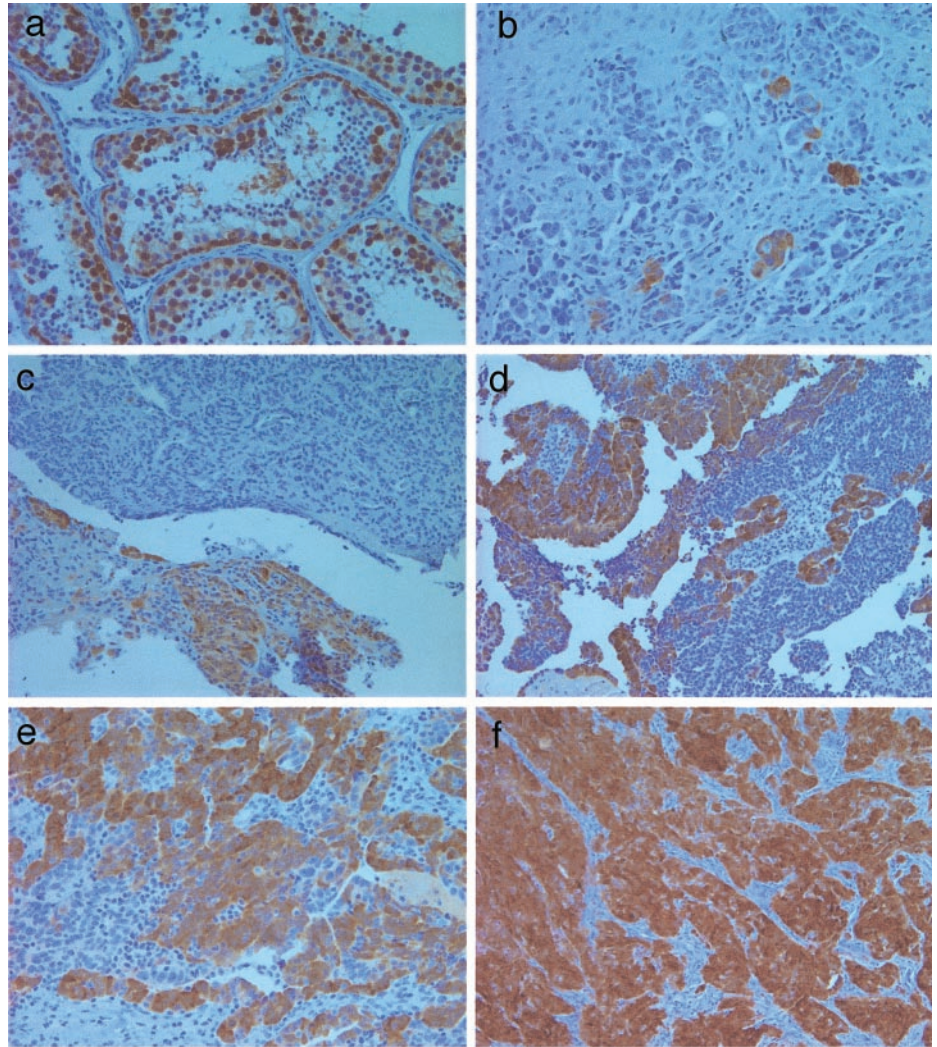


Fig. 2. *a-f*, immunohistochemical staining of NY-ESO-1 antigen with mAb ES 121. Magnification: $\times 20$ for normal testis (*a*) and $\times 10$ for tumor tissue (*b-f*). *a*, seminiferous tubules with strong intratubular staining of mostly early spermatogenic cells, no reactivity with spermatids or Sertoli cell, negative interstitial tissue. *b-f*, ES121 staining of serous papillary EOC showing focal, +, ++, +++, or +++++ immunoreactivity patterns.

had a median disease-free survival of 32 months (CI, 17–47 months) and a median overall survival of 54 months (CI, 29–80 months), compared with 32 (CI, 18–46) and 55 (CI, 40–70) months, respectively, among patients whose tumors did not express NY-ESO-1 ($P = 0.74$ and 0.97 , respectively, for both comparisons). There was no statistically significant difference in the distribution of the expression of NY-ESO-1 and histological grade ($P = 0.21$). Although tissue expression of NY-ESO-1 was associated with a higher stage of disease ($P = 0.005$), the estimated 5-year disease-free survival rate was 30% (CI, 12%–48%) among patients whose tumors expressed NY-ESO-1 and 33% (CI, 11%–57%) among patients whose tumors did not express NY-ESO-1 (not significant; Fig. 3*a*). The estimated 5-year overall survival rate was 47% (CI, 33%–61%) among patients whose tumors expressed NY-ESO-1 and 44% (CI, 30%–58%) among patients whose tumors did not express NY-ESO-1 (not significant; Fig. 3*b*). There were no significant differences in survival distributions when patients were compared based on the degree of tissue immunoreactivity ($P = 0.06$; Table 4).

In the analysis of patients whose tumors expressed LAGE-1, antigen expression in EOC tissues was also associated with higher stage of disease ($P = 0.006$). However, there were no significant differences in the distribution of LAGE-1 expression and histological grade, disease-free survival, and overall survival (Table 3). When all patients with tissue expression of either NY-ESO-1 and/or LAGE-1 by RT-PCR and/or IHC were considered [*i.e.*, 55 of 107 (51%)], no signif-

icant correlation was observed with stage, grade, histology, disease-free survival, or overall survival.

Among the 97 patients who demonstrated complete response to front-line surgery and chemotherapy, 36 (37%) patients demonstrated tissue expression of NY-ESO-1 by RT-PCR and/or IHC. There were no significant differences in disease-free and overall survival when this group was stratified by NY-ESO-1 expression. A subset of 62 patients with complete response to front-line surgery and chemotherapy were compared by LAGE-1 mRNA expression. Again, no significant differences in disease-free and overall survival were noted between the groups.

Antibody Response to NY-ESO-1/LAGE-1 in Ovarian Cancer Patients. A total of 171 serum samples from 48 patients were analyzed by ELISA for NY-ESO-1/LAGE-1 antibodies. These sera consisted of preoperative and serial specimens obtained during patients' course of disease (range, 1–3 years). Because of the high degree of homology between the NY-ESO-1 and LAGE-1, the serological assay did not reliably distinguish NY-ESO-1 and LAGE-1 in human sera. Therefore, the antibody responses to both antigens were considered together. A demonstrable antibody response to NY-ESO-1/LAGE-1 was found in 12 (25%) of the patients. One patient developed NY-ESO-1/LAGE-1 antibody 7 months after diagnosis and remained positive 3 years after initial therapy. All of the remaining patients antibodies present at the time of diagnosis. Another NY-ESO-1/LAGE-1 antibody-positive patient at baseline became negative 1 year

Table 4 NY-ESO-1 expression by IHC (mAb ES121) and correlation with clinicopathological characteristics in EOC

Clinicopathological characteristics	Negative	Positive	Focal ^a	+	++	+++	++++
All tumors, n (%) (n = 142)	80	62 (43%)	5 (3.5%)	20 (14%)	16 (11.2%)	18 (12.6%)	3 (2.1%)
Tumor grade, n							
G1	6	2			2		
G2	6	1			1		
G3	68	59	5	20	13	18	3
FIGO stage, n							
IA	2	1		1			
IC	2						
IIB	2	1		1			
IIC	5						
IIIA	1						
IIIB	3						
IIIC	58	54	3	18	14	16	3
IV	7	6	2		2	2	
Histology (n)							
Serous	60	51	4	17	12	15	3
Clear cell	3	2		1		1	
Endometrioid	4	1			1		
Mucinous	4						
Undifferentiated	2	2			1	1	
Others	7	6	1	2	2	1	
Dead of disease, ^b n (%)	42 (52%)	26 (42%)		8 (40%)	8 (50%)	8 (44%)	2 (67%)

^a Focal, <5%; +, <25%; ++, >25–50%; +++, >50–75%; +++, >75%.

^b Median survival for patients with NY-ESO-1-positive and -negative tumors was 54 (CI, 26–83) and 45 (CI, 34–55) months, respectively (P = 0.30). Percentages were calculated by the proportion of patients dead of disease in relation to the total number of patients for each column.

after frontline treatment. Subsequently, she developed recurrent disease after 4 months and again became NY-ESO-1 and LAGE-1 antibody positive. All NY-ESO-1 or LAGE-1 antibody-positive patients at baseline and with sera available at up to 3 years of follow-up continued to demonstrate the presence of antibody.

Correlation of Antibody Response to NY-ESO-1/LAGE-1 with Clinical Outcome. Among the 48 patients tested by ELISA, 37 had NY-ESO-1/LAGE-1-expressing tumors (by RT-PCR and/or IHC). NY-ESO-1/LAGE-1 antibody was present in 11 of 37 (30%) EOC patients with NY-ESO-1/LAGE-1-expressing tumors. Only one pa-

tient whose tumor did not express NY-ESO-1 or LAGE-1 had demonstrable antibody to NY-ESO-1/LAGE-1. The analysis of clinical outcome comparing the 11 NY-ESO-1/LAGE-1 antibody-positive with the 27 antibody-negative patients with NY-ESO-1/LAGE-1-expressing tumors showed no significant differences in disease-free and overall survival. The detailed characteristics of the 12 patients with demonstrable NY-ESO-1/LAGE-1 antibody are shown in Table 6. All of the patients had tumors of serous histology (except one patient with transitional cell carcinoma) and advanced stage (11 with stage IIIC, 1 with stage IV disease). The analysis of serum samples obtained at 3 years on all eight patients with extended follow-up, including patients who remained disease-free after initial therapy, demonstrated the presence of NY-ESO-1/LAGE-1 antibody.

Table 5 Correlation between NY-ESO-1 expression (mRNA and/or IHC) and clinicopathological features in EOC

Pathological and clinical features	NY-ESO-1 positive	NY-ESO-1 negative
All tumors (n = 190)	82 (43.2%)	108 (56.8%)
Age, median (range), years	62 (22–84)	59 (26–89)
Tumor grade (n)		
G1	2	9
G2	5	5
G3	75	94
FIGO stage (n)		
Ia	1	4
Ib		1
Ic	1	3
IIB	1	3
IIC		7
IIIA		
IIIB		4
IIIC	71	78
IV	7	8
Histology (n)		
Papillary serous	65	78
Clear cell	2	6
Endometrioid	1	7
Mucinous	2	2
Undifferentiated	2	4
Others (transitional, mixed, carcinosarcoma)	7	6
Response, n (%)		
Complete response	36 (44%)	61 (51%)
Partial response	42 (51%)	39 (36%)
Total Recurrences/persistent disease, ^a n (%)	67 (82%)	71 (66%)
Dead of disease, n (%)	35 (43%)	42 (39%)
Alive with disease, n (%)	31 (38%)	28 (36%)
Alive with no evidence of disease, n (%)	16 (20%)	37 (34%)

^a Median disease-free survival (excluding patients with persistent disease) for patients with NY-ESO-1-negative and -positive tumors: 32 (18–46) and 32 (17–47) months, respectively (P = 0.74).

DISCUSSION

One of the major barriers to antigen-specific immunotherapy in EOC is the lack of well-defined immunogenic tumor antigens. The need to identify and characterize tumor antigens in EOC has become even more compelling because of recent evidence demonstrating that the presence of intratumoral T cells correlates with improved clinical outcome (31), suggesting that efforts to stimulate and/or augment the antitumor immune response are likely to be of particular benefit in this disease. To assess the utility of two of the CT antigens as targets for specific immunotherapy of EOC, the present comprehensive analysis of NY-ESO-1 and LAGE-1 was undertaken on a large panel of ovarian tumors and cell lines. Our results indicate aberrant expression of NY-ESO-1 and LAGE-1 in 43% and 21% of EOC specimens, respectively. In addition, we found composite expression of NY-ESO-1 and LAGE-1 in 11% of EOC specimens. The frequency of NY-ESO-1 expression in EOC that we report is generally higher than reported for the majority of other tumors, except for synovial sarcoma (32). For example, expression of NY-ESO-1 mRNA has been found in 25–30% of several tumor types, including melanoma (27), esophageal carcinoma (33), and bladder cancer (27). With regard to LAGE-1, the expression frequencies reported for other tumor sites are 29% in melanoma (22), 44% in bladder cancer (22), 33% in lung carcinoma (22), and 39% in esophageal cancer (33). Considering the expression of either NY-ESO-1 or LAGE-1 in EOC specimens in our study, ~50% showed expression of at least one of these two CT

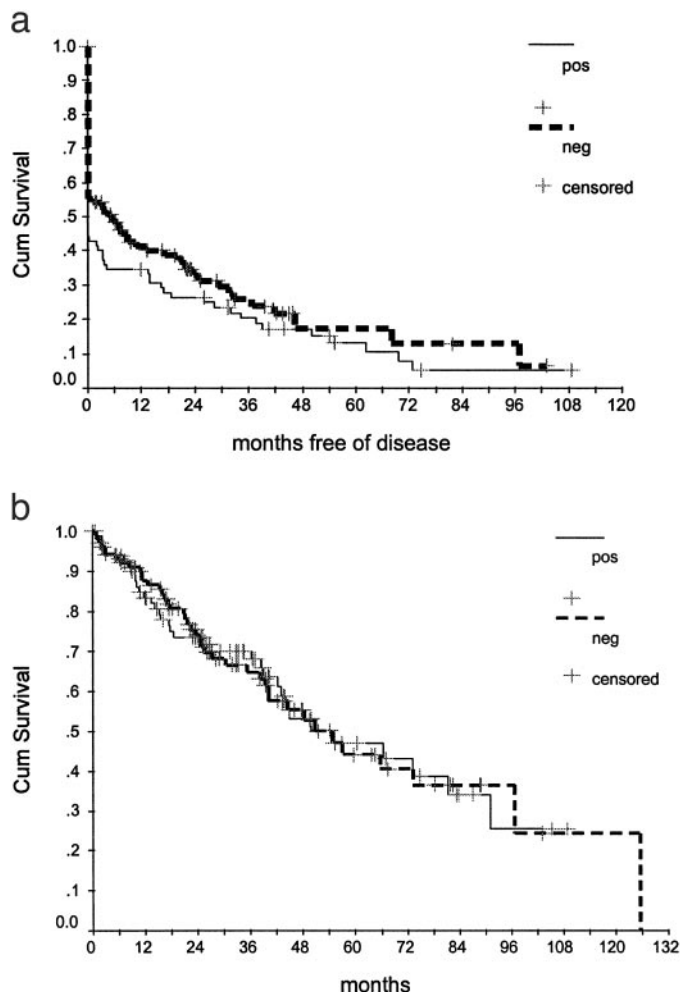


Fig. 3. *a*, Kaplan-Meier estimates of disease-free survival in EOC patients according to tumor expression of NY-ESO-1 by RT-PCR and/or IHC. *b*, Kaplan-Meier estimates of overall survival in EOC patients according to tumor expression of NY-ESO-1 by RT-PCR and/or IHC. *Cum*, cumulative

antigens. These findings suggest that NY-ESO-1 and LAGE-1 might represent targets for immunotherapy in a significant proportion of patients with EOC.

In an effort to determine the inherent immunogenicity of NY-

ESO-1 and LAGE-1 in patients with EOC, we studied a subset of 48 patients for whom tumor and serum specimens from the same patients were available. We compared the NY-ESO-1 and LAGE-1 antibody status with mRNA and/or protein expression in the autologous tumor. The results showed that NY-ESO-1/LAGE-1 antibodies were present in 30% of patients with NY-ESO-1- and LAGE-1-positive tumors, and only one patient with an NY-ESO-1- and LAGE-1-negative tumor had NY-ESO-1 antibody. In a survey of sera from normal individuals and cancer patients, Stockert *et al.* (25) reported the presence of antibodies to NY-ESO-1 in 40–50% of patients with advanced NY-ESO-1-expressing tumors, and no patients with NY-ESO-1-negative tumors had NY-ESO-1 antibody. Although the reported frequency of antibody response was only 12.5% in ovarian cancer patients in the study (25), the tumor NY-ESO-1/LAGE-1 antigen phenotype was not known.

Our demonstration of humoral immune response in a significant proportion of EOC patients with NY-ESO-1/LAGE-1-expressing tumors is consistent with the known immunogenicity of these antigens and the biology of EOC. This is particularly relevant in light of previous reports demonstrating that a humoral immune response to NY-ESO-1 was predictive of a strong CD8⁺ T-cell response to NY-ESO-1-derived peptides, as measured by tetramer, enzyme-linked immunospot, and cytotoxicity analyses (34). Of interest is our finding of a spontaneous humoral immune response persisting for several years in EOC patients with no apparent evidence of disease. Although it is possible that long-lasting spontaneous immunity to NY-ESO-1/LAGE-1 can occur in tumor-free individuals, an alternative hypothesis is that these patients continue to have micrometastatic disease that provides the antigenic stimulus for the immune system. This latter explanation is particularly appealing because the vast majority of patients with EOC will ultimately have recurrences, and previous reports indicate that the humoral immune response to NY-ESO-1 is antigen driven (25).

Previous studies have shown that patients with NY-ESO-1 antigen expression and antibody tend to have advanced-stage cancer. For example, a higher frequency of NY-ESO-1 expression in bladder cancer was correlated with high nuclear grade (35), and NY-ESO-1 antibody response was correlated with advanced stage of disease (25, 36). In our present study of a large set of EOC patients, the lack of correlation of NY-ESO-1 and LAGE-1 antigen expression with clinicopathological characteristics (histological type, tumor grade, recurrence, and survival) may reflect the fact that 96% of the patients (Table 1) had advanced stage disease. In a recent study of patients

Table 6 Characteristics of NY-ESO-1/LAGE-1 antibody-positive EOC patients^a

Patient	Stage	Tumor status		Initial clinical response	Recurrence ^d	Follow-up duration (months)	Current status
		NY-ESO-1 ^b	LAGE-1 ^c				
1	IIIC	Negative	Negative	CR ^e	No	12	ANED
2	IIIC	Positive	Positive	CR	No	44	ANED
3	IIIC	Positive	Positive	CR	Yes	36	AWD
4	IIIC	Positive	NA	PR	P	15	DOD
5	IV	Positive	Positive	PR	P	36	AWD
6	IIIC	Positive	Negative	CR	No	16	ANED
7	IIIC	Positive	Negative	PR	P	16	AWD
8	IIIC	Positive	NA	CR	Yes	40	AWD
9	IIIC	Positive	NA	CR	No	46	ANED
10	IIIC	Positive	NA	CR	Yes	92	ANED
11	IIIC	Positive	Negative	CR	Yes	96	ANED
12	IIIC	Positive	NA	CR	Yes	90	DOD

^a All tumors tissues were serous and grade 3 histology, except tumor from patient 8, which was transitional cell histology. All patients had stage IIIC disease, except patient 5, with stage IV disease.

^b NY-ESO-1 expression was determined by RT/PCR and/or IHC.

^c LAGE-1 expression was determined by RT-PCR.

^d Response to frontline surgery and chemotherapy.

^e CR, complete response; ANED, alive, no evidence of disease; AWD, alive with disease; NA, undetermined LAGE-1 status; PR, partial response; P, persistence/progression; DOD, dead of disease.

with early stage EOC, low frequencies of NY-ESO-1 expression were detected,⁴ suggesting that the expression of NY-ESO-1 also correlates with advanced stage EOC. This raises important questions about the role of CT antigens in tumorigenesis, invasion and metastasis in EOC. First, what are the functions of these CT antigens? Second, at what stage in the malignant process does CT antigen expression become evident? Third, given the immunogenicity of some the CT antigens (e.g., NY-ESO-1), does the presence of detectable spontaneous immune response correlate with better clinical outcome? Fourth, will the induction and/or augmentation of CT antigen-specific immunity by immunotherapy likely to be associated with clinical benefit? Because of the small number of patients with spontaneous immunity to NY-ESO-1 in the present study, it is not possible to make a definitive statement about the prognostic significance of NY-ESO-1 immunity in EOC.

Even if the expression of CT antigens in tumors represents only an epiphenomenon rather than playing a clearly defined role in tumor progression in EOC, CT antigens still represent attractive targets for immunotherapy because of their tissue-restricted expression and immunogenicity. For example, in a recent Phase I clinical trial, 12 HLA-A2⁺ patients with progressing NY-ESO-1-expressing metastatic tumors of different types were vaccinated intradermally with NY-ESO-1 peptides first alone and then in combination with granulocyte-macrophage colony-stimulating factor as a systemic adjuvant (37). In five of seven vaccinated patients who were initially NY-ESO-1 antibody negative, individual metastases stabilized or regressed after induction of NY-ESO-1-specific CD8⁺ T-cell responses. In addition, there was disease stabilization after NY-ESO-1 immunization in three of five antibody-positive patients, indicating that vaccination may also yield clinical benefit in patients with baseline spontaneous immunity to NY-ESO-1.

We conclude that NY-ESO-1 and LAGE-1 antigens meet the criteria for inclusion as targets for active specific immunotherapy in EOC. These criteria include (a) demonstration that the tumor antigen is expressed at a reasonable frequency in tumors, (b) demonstration of restricted or no expression in other normal tissues, and (c) demonstration of immunogenicity. On the basis of these considerations, we have initiated a clinical trial of antigen-specific immunotherapy in EOC, using these target CT antigens. However, the fact that NY-ESO-1 and LAGE-1 are expressed in only a fraction of EOC underlines the need for identifying other antigens in this disease that could serve as targets for immunotherapy. In this regard, we have continued the analysis of additional CT antigens in EOC and initiated a comprehensive "immunomic" analysis (38) of EOC to uncover additional antigenic targets for polyvalent vaccine development.

REFERENCES

- Greenlee, R. T., Hill-Harmon, M. B., Murray, T., and Thun, M. Cancer statistics, 2001. *CA Cancer J. Clin.*, *51*: 15–36, 2001.
- McGuire, W. P., Hoskins, W. J., Brady, M. F., Kucera, P. R., Partridge, E. E., Look, K. Y., Clarke-Pearson, D. L., and Davidson, M. Cyclophosphamide and cisplatin versus paclitaxel and cisplatin: a Phase III randomized trial in patients with suboptimal stage III/IV ovarian cancer (from the Gynecological Oncology Group). *Semin. Oncol.*, *23*: 40–47, 1996.
- Carey, T. E., Takahashi, T., Resnick, L. A., Oettgen, H. F., and Old, L. J. Cell surface antigens of human malignant melanoma: mixed hemadsorption assays for humoral immunity to cultured autologous melanoma cells. *Proc. Natl. Acad. Sci. USA*, *73*: 3278–3282, 1976.
- Knuth, A., Danowski, B., Oettgen, H. F., and Old, L. J. T-cell-mediated cytotoxicity against autologous malignant melanoma: analysis with interleukin 2-dependent T-cell cultures. *Proc. Natl. Acad. Sci. USA*, *81*: 3511–3515, 1984.
- van der Bruggen, P., Traversari, C., Chomez, P., Lurquin, C., De Plaen, E., Van den Eynde, B., Knuth, A., and Boon, T. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. *Science (Wash. DC)*, *254*: 1643–1647, 1991.
- Sahin, U., Tureci, O., Schmitt, H., Cochlovius, B., Johannes, T., Schmits, R., Stenner, F., Luo, G., Schobert, I., and Pfreundschuh, M. Human neoplasms elicit multiple specific immune responses in the autologous host. *Proc. Natl. Acad. Sci. USA*, *92*: 11810–11813, 1995.
- Brichard, V., Van Pel, A., Wolfel, T., Wolfel, C., De Plaen, E., Lethe, B., Coulic, P., and Boon, T. The tyrosinase gene codes for an antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. *J. Exp. Med.*, *178*: 489–495, 1993.
- Coulic, P. G., Brichard, V., Van Pel, A., Wolfel, T., Schneider, J., Traversari, C., Mattei, S., De Plaen, E., Lurquin, C., Szikora, J. P., et al. A new gene coding for a differentiation antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. *J. Exp. Med.*, *180*: 35–42, 1994.
- Kawakami, Y., Eliyahu, S., Delgado, C. H., Robbins, P. F., Rivoltini, L., Topalian, S. L., Miki, T., and Rosenberg, S. A. Cloning of the gene coding for a shared human melanoma antigen recognized by autologous T cells infiltrating into tumor. *Proc. Natl. Acad. Sci. USA*, *91*: 3515–3519, 1994.
- Kawakami, Y., Eliyahu, S., Jennings, C., Sakaguchi, K., Kang, X., Southwood, S., Robbins, P. F., Sette, A., Appella, E., and Rosenberg, S. A. Recognition of multiple epitopes in the human melanoma antigen gp100 by tumor-infiltrating T lymphocytes associated with *in vivo* tumor regression. *J. Immunol.*, *154*: 3961–3968, 1995.
- Wolfel, T., Hauer, M., Schneider, J., Serrano, M., Wolfel, C., Klehmann-Hieb, E., De Plaen, E., Hankeln, T., Meyer zum Buschenfelde, K. H., and Beach, D. A p16INK4a-insensitive CDK4 mutant targeted by cytolytic T lymphocytes in a human melanoma. *Science (Wash. DC)*, *269*: 1281–1284, 1995.
- Robbins, P. F., El-Gamil, M., Li, Y. F., Kawakami, Y., Loftus, D., Appella, E., and Rosenberg, S. A. A mutated β -catenin gene encodes a melanoma-specific antigen recognized by tumor infiltrating lymphocytes. *J. Exp. Med.*, *183*: 1185–1192, 1996.
- Mandruzzato, S., Brasseur, F., Andry, G., Boon, T., and van der Bruggen, P. A CASP-8 mutation recognized by cytolytic T lymphocytes on a human head and neck carcinoma. *J. Exp. Med.*, *186*: 785–793, 1997.
- Scanlan, M. J., Chen, Y. T., Williamson, B., Gure, A. O., Stockert, E., Gordan, J. D., Tureci, O., Sahin, U., Pfreundschuh, M., and Old, L. J. Characterization of human colon cancer antigens recognized by autologous antibodies. *Int. J. Cancer*, *76*: 652–658, 1998.
- Cheever, M. A., Disis, M. L., Bernhard, H., Gralow, J. R., Hand, S. L., Huseby, E. S., Qin, H. L., Takahashi, M., and Chen, W. Immunity to oncogenic proteins. *Immunol. Rev.*, *145*: 33–59, 1995.
- Gnjatic, S., Cai, Z., Viguier, M., Chouaib, S., Guillet, J. G., and Choppin, J. Accumulation of the p53 protein allows recognition by human CTL of a wild-type p53 epitope presented by breast carcinomas and melanomas. *J. Immunol.*, *160*: 328–333, 1998.
- Jager, D., Stockert, E., Scanlan, M. J., Gure, A. O., Jager, E., Knuth, A., Old, L. J., and Chen, Y. T. Cancer-testis antigens and ING1 tumor suppressor gene product are breast cancer antigens: characterization of tissue-specific ING1 transcripts and a homologue gene. *Cancer Res.*, *59*: 6197–6204, 1999.
- Tindle, R. W. Human papillomavirus vaccines for cervical cancer. *Curr. Opin. Immunol.*, *8*: 643–650, 1996.
- Lenette, E. T., Winberg, G., Yadav, M., Enblad, G., and Klein, G. Antibodies to LMP2A/2B in EBV-carrying malignancies. *Eur. J. Cancer*, *31A*: 1875–1878, 1995.
- Boon, T., and van der Bruggen, P. Human tumor antigens recognized by T lymphocytes. *J. Exp. Med.*, *183*: 725–729, 1996.
- Chen, Y. T., Boyer, A. D., Viars, C. S., Tsang, S., Old, L. J., and Arden, K. C. Genomic cloning and localization of CTAG, a gene encoding an autoimmunogenic cancer-testis antigen NY-ESO-1, to human chromosome Xq28. *Cytogenet. Cell Genet.*, *79*: 237–240, 1997.
- Lethe, B., Lucas, S., Michaux, L., De Smet, C., Godelaine, D., Serrano, A., De Plaen, E., and Boon, T. LAGE-1, a new gene with tumor specificity. *Int. J. Cancer*, *76*: 903–908, 1998.
- Chen, Y. T., Scanlan, M. J., Sahin, U., Tureci, O., Gure, A. O., Tsang, S., Williamson, B., Stockert, E., Pfreundschuh, M., and Old, L. J. A testicular antigen aberrantly expressed in human cancers detected by autologous antibody screening. *Proc. Natl. Acad. Sci. USA*, *94*: 1914–1918, 1997.
- Jager, E., Stockert, E., Zidianakis, Z., Chen, Y. T., Karbach, J., Jager, D., Arand, M., Ritter, G., Old, L. J., and Knuth, A. Humoral immune responses of cancer patients against "Cancer-Testis" antigen NY-ESO-1: correlation with clinical events. *Int. J. Cancer*, *84*: 506–510, 1999.
- Stockert, E., Jager, E., Chen, Y. T., Scanlan, M. J., Gout, I., Karbach, J., Arand, M., Knuth, A., and Old, L. J. A survey of the humoral immune response of cancer patients to a panel of human tumor antigens. *J. Exp. Med.*, *187*: 1349–1354, 1998.
- Serov, S. F., Scully, R. E., and Sobin, L. H. Histological typing of ovarian tumors. *In: International Classification of Tumors*, Vol. 9. Geneva: WHO, 1973.
- Jungbluth, A. A., Chen, Y. T., Stockert, E., Busam, K. J., Kolb, D., Iversen, K., Coplan, K., Williamson, B., Altorki, N., and Old, L. J. Immunohistochemical analysis of NY-ESO-1 antigen expression in normal and malignant human tissues. *Int. J. Cancer*, *92*: 856–860, 2001.
- SPSS Inc., *Spss Anc. SPSS Advanced Statistics*, 7.5 ed. Chicago: SPSS, 1997.
- Kaplan, E. L., and Meier, P. Nonparametric estimation from incomplete observations. *J. Am. Stat. Assoc.*, *53*: 457–486, 1958.
- Cox, D. R. Regression models and life-tables. *J. R. Stat. Soc.*, *34*: 187–220, 1972.
- Zhang, L., Conejo-Garcia, J. R., Katsaros, D., Gimotty, P. A., Massobrio, M., Regnani, G., Makrigiannakis, A., Gray, H., Schlienger, K., Liebman, M. N., Rubin, S. C., and Coukos, G. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. *N. Engl. J. Med.*, *348*: 203–213, 2003.
- Jungbluth, A. A., Antonescu, C. R., Busam, K. J., Iversen, K., Kolb, D., Coplan, K., Chen, Y. T., Stockert, E., Ladanyi, M., and Old, L. J. Monophasic and biphasic synovial sarcoma abundantly express cancer/testis antigen NY-ESO-1 but not MAGE-A1 or CT7. *Int. J. Cancer*, *94*: 252–256, 2001.

⁴ A. A. Jungbluth, personal communication.

33. Mashino, K., Sadanaga, N., Tanaka, F., Yamaguchi, H., Nagashima, H., Inoue, H., Sugimachi, K., and Mori, M. Expression of multiple cancer-testis antigen genes in gastrointestinal and breast carcinomas. *Br. J. Cancer*, *85*: 713–720, 2001.
34. Jager, E., Nagata, Y., Gnjatic, S., Wada, H., Stockert, E., Karbach, J., Dunbar, P. R., Lee, S. Y., Jungbluth, A., Jager, D., Arand, M., Ritter, G., Cerundolo, V., Dupont, B., Chen, Y. T., Old, L. J., and Knuth, A. Monitoring CD8 T cell responses to NY-ESO-1: correlation of humoral and cellular immune responses. *Proc. Natl. Acad. Sci. USA*, *97*: 4760–4765, 2000.
35. Kurashige, T., Noguchi, Y., Saika, T., Ono, T., Nagata, Y., Jungbluth, A., Ritter, G., Chen, Y. T., Stockert, E., Tsushima, T., Kumon, H., Old, L. J., and Nakayama, E. Ny-ESO-1 expression and immunogenicity associated with transitional cell carcinoma: correlation with tumor grade. *Cancer Res.*, *61*: 4671–4674, 2001.
36. Wang, R. F., Johnston, S. L., Zeng, G., Topalian, S. L., Schwartzentruber, D. J., and Rosenberg, S. A. A breast and melanoma-shared tumor antigen: T cell responses to antigenic peptides translated from different open reading frames. *J. Immunol.*, *161*: 3598–3606, 1998.
37. Jager, E., Gnjatic, S., Nagata, Y., Stockert, E., Jager, D., Karbach, J., Neumann, A., Rieckenberg, J., Chen, Y. T., Ritter, G., Hoffman, E., Arand, M., Old, L. J., and Knuth, A. Induction of primary NY-ESO-1 immunity: CD8+ T lymphocyte and antibody responses in peptide-vaccinated patients with NY-ESO-1+ cancers. *Proc. Natl. Acad. Sci. USA*, *97*: 12198–12203, 2000.
38. Lee, S. Y., Obata, Y., Yoshida, M., Stockert, E., Williamson, B., Jungbluth, A. A., Chen, Y. T., Old, L. J., and Scanlan, M. J. Immunomic analysis of human sarcoma. *Proc. Natl. Acad. Sci. USA*, *100*: 2651–2656, 2003.

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NY-ESO-1 and LAGE-1 Cancer-Testis Antigens Are Potential Targets for Immunotherapy in Epithelial Ovarian Cancer

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