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Expression of MAGE-A and NY-ESO-1 in Primary and Metastatic Cancers

Tristen S. Park^{*}, Eric M. Groh^{*}, Krishna Patel^{*}, Sid P. Kerkar[†], Chyi-Chia Richard Lee[†], and Steven A. Rosenberg^{*}

^{*}Surgery Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD.

[†]Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD.

Summary:

Melanoma-associated antigen-A (MAGE-A) and New York esophageal squamous cell cancer-1 (NY-ESO-1) are 2 cancer testis antigens (CTA) demonstrating potential for use in targeted immunotherapy. Clinical trials in melanoma and synovial sarcomas targeting these antigens in immune-based therapies have demonstrated durable tumor regression. Although protein expression of NY-ESO-1 has been assessed in a variety of cancer types, the expression of MAGE-A has not been studied in depth. In this study we analyzed MAGE-A and NY-ESO-1 expression in 314 melanoma specimens from 301 melanoma patients, 38 patients with squamous cell cancers and 111 patients with adenocarcinomas. Our results demonstrated higher expression of MAGE-A compared with NY-ESO-1 in melanomas (32% vs. 13%) and squamous cell carcinomas (45% vs. 7.9%), and higher expression of both CTAs in metastatic versus primary tumors. CTA expression in adenocarcinomas was low (MAGE-A: 10%, NY-ESO-1: 0.9%). In addition, we looked at concordance of expression among metastatic melanoma lesions within the same patient and found concordant expression in 38 of 47 patients for MAGE-A and 43 of 47 patients for NY-ESO-1. Our study demonstrated that the MAGE-A family may be of greater utility than NY-ESO-1 for targeted immune-therapy in a variety of cancer histologies, in particular metastatic melanomas and squamous cell carcinomas.

Keywords

MAGE-A; NY-ESO-1; melanoma; breast cancer; squamous cell cancer; cancer testis antigen

Immunotherapy has been shown to be a promising therapeutic option for metastatic cancers of multiple histologies.¹ Cancer testis antigens (CTAs) have been explored as therapeutic targets for immunotherapy. Although CTAs are expressed in a variety of epithelial cancers, their expression is often limited in normal adult tissues to the testes, whose cells do not

Reprints: Tristen Park, Surgery Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892 (s.tristen.park@gmail.com).

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express HLA molecules and are therefore not susceptible to T-cell recognition.² CTAs are expressed in a variety of human malignancies including melanoma and carcinomas of bladder, lung, liver, breast, ovary, and synovial sarcomas.^{3–13}

Direct targeting of human tumors using autologous tumor infiltrating lymphocytes have demonstrated an objective response rate of 50%–70% with the addition of preconditioning nonmyeloablative lymphodepleting chemotherapy.¹⁴ The use of genetically modified T cells to target a specific marker has further expanded the ability to treat patients with metastatic cancer in both liquid and solid tumors.¹ Two CTAs that have been targeted by TCR gene therapy include melanoma-associated antigen-A (MAGE-A) and New York esophageal squamous cell cancer-1 (NY-ESO-1) and have shown objective responses in clinical trials in melanoma and synovial cell sarcoma patients.^{15,16}

Studies have also shown that in multiple histologies, higher CTA expression is indicative of poor prognosis and more advanced stage. NY-ESO-1 protein expression was found to be higher in more aggressive histologies among primary melanomas.⁵ CTA expression has been demonstrated to be higher in metastatic lesions versus primary lesions.^{3,5,8} Therefore, targeted immunotherapy using these antigens may be more efficacious in the metastatic setting rather than in localized disease.

MAGE-A has been shown to be overexpressed in a variety of malignancies, as assessed by gene expression arrays; however, series utilizing immunohistochemistry (IHC) are few.^{9,17} NY-ESO-1 has been more widely studied and IHC expression has been reported in urothelial,⁴ esophageal,⁷ non-small cell carcinomas,¹¹ ovarian,⁶ and head and neck carcinomas.^{6,11} High levels of NY-ESO-1 protein expression in melanoma (30%) and synovial sarcomas (75%) have been reported.^{8,13} Other cancer histologies remain to be assessed at the protein level for expression of MAGE-A and NY-ESO-1.

In this study we evaluated the expression of NY-ESO-1 and MAGE-A by IHC in 314 melanoma lesions from 301 patients, 38 squamous cell cancers and 111 common adenocarcinomas evaluated in the Surgery Branch, National Cancer Institute. We found MAGE-A to be more highly expressed than NY-ESO-1 in melanomas and squamous cell carcinomas, and expression of both to be low in common adenocarcinomas. In addition we studied multiple metastatic melanoma lesions from the same patient to establish the concordance of NY-ESO-1 or MAGE-A expression among lesions. These data suggest that targeting MAGE-A may provide more benefit than NY-ESO-1 in melanomas and squamous cell cancers and that high expression in metastatic lesions versus primary lesions suggests that targeted treatment in the metastatic setting may be of more benefit.

METHODS

Case Selection

A series of 40 primary and 274 metastatic melanoma specimens from 301 patients, 18 primary and 20 metastatic lesions from 38 squamous cell patients, and 56 primary and 55 metastatic lesions from 111 adenocarcinomas patients evaluated by the Surgery Branch, National Cancer Institute between April 2008 and 2015 were retrieved from the archives of

the Laboratory of Pathology at the National Cancer Institute. All studies were carried out in accordance with the approved guidelines of the National Cancer Institute Institutional Review Board. Cases with available unstained slides were selected, and all were reviewed histologically to confirm the diagnosis.

Tumor IHC

Most cases were studied by automated IHC using Leica Bond Max automation with Leica refine detection kit. The primary antibodies used were NY-ESO-1 (1:100 dilution; Invitrogen/Life Technologies, Grand Island, NY) and MAGE-A (1:100 dilution; Santa-Cruz Biotech, Dallas, TX). Heat-induced epitope retrieval was used for 25 minutes (Leica high-pH buffer), followed by incubation of primary antibody at room temperature (30 min). This was followed by Leica polymer (15 min), postpolymer (15 min), peroxide block, diaminobenzidine (10 min), and hematoxylin counterstain. A small number of cases were studied using Ventana Benchmark Ultra automation with the same primary antibodies and reagents associated with that automation, including high pH epitope retrieval buffer.

NY-ESO-1 and MAGE staining intensity was assessed by at least 2 pathologists and was scored as positive or negative depending on the clear presence of tumor cell staining that was cytoplasmic for NY-ESO and cytoplasmic and/or nuclear for MAGE-A. Intensity of staining was also assessed and both weak and strong intense staining was considered positive. The percentage of staining neoplastic cells was estimated for each case and sections showing 50% of the tumor cells staining positive with any intensity were categorized as positive for our study.

Statistics

All statistical analysis was performed using a 2-tailed Fischer exact test.

RESULTS

Expression of MAGE-A and NY-ESO-1 in Primary and Metastatic Melanomas

In total, 314 tumors from 301 patients were evaluated for MAGE-A and NY-ESO-1 expression including 40 primary lesions and 274 metastatic lesions. There were 13 patients where both primary and metastatic lesions were evaluated. MAGE-A expression was positive in 3 of 40 (7.5%) primary melanomas and 98 of 274 (36%) of meta-static melanomas (Table 1). Thus, MAGE-A expression in metastatic sites was higher compared with primary lesions ($P=0.0002$). NY-ESO-1 expression was positive in 2 of 40 (5%) of primary melanomas and 38 of 274 (14%) of metastatic melanomas ($P=0.13$) (Table 1). Although 47 of the 301 patients were found to have >1 metastatic lesion in our study, we only included the first metastatic lesion resected per patient for the analysis as shown above.

Representative slides of positive MAGE-A and NYESO-1 staining in melanoma lesions are shown in Figure 1.

Expression of MAGE-A and NY-ESO-1 in Melanoma Patients With Multiple (>1) Metastatic Lesions

We assessed for concordance of staining between metastatic lesions within the same patient (Table 2) in the 47 patients with >1 metastatic lesions. We found that for MAGE-A, 20 of 47 had negative staining for all lesions. Nine of the 47 patients had discordant staining between their metastatic lesions and 18 of the 47 had positive staining in all their metastatic sites per patient. NY-ESO-1 staining was negative for all lesions in 41 of the 47 patients, 4 of the 47 patients had mixed expression and 2 of the 47 patients had positive staining in all their metastatic sites.

MAGE-A3 and NY-ESO-1 Expression in Melanoma Patients With Paired Primary and Metastatic Lesions

Thirteen patients in the study had both a primary and metastatic lesion assessed for MAGE-A3 and NY-ESO-1 (Table 3). Eight cases showed negative expression of MAGE-A3 in both primary and metastatic lesions, 5 cases showed positive staining in a metastatic site and negative staining in the primary site from the same patient. There were no patients who had positive expression of MAGE-A3 in a primary site and negative expression in paired metastatic site.

For NY-ESO-1, 11 patients showed negative staining in both paired primary and metastatic sites. One patient showed positive expression in a metastatic site and negative expression in a primary site. One patient showed negative expression in a metastatic site and positive expression in a primary site.

Expression of MAGE-A and NY-ESO-1 in Primary and Metastatic Melanomas From Various Organ Sites

MAGE expression was seen in most organ sites evaluated (Table 4). Gastrointestinal (GI) metastases demonstrated MAGE-A positivity in 11 of 15 patients. In addition, MAGE-A positivity was seen in 23 of 80 lymph node metastases, 22 of 53 soft tissue metastases, and 17 of 42 patients with metastatic skin involvement. Of note brain metastases were positive for MAGE-A in all patients (n = 4) but clinical significance is unclear due to small sample size. MAGE-A was not seen in salivary metastases and kidney metastases, however, the sample size was small.

NY-ESO-1 positivity was less compared with MAGE-A positivity. GI metastases were positive for NY-ESO-1 expression in 4 of 15 patients. Nine of 53 soft tissue metastases were positive for NY-ESO-1. No positivity was seen in muscle, salivary, spleen, genitourinary, and kidney metastases but clinical significance is unclear due to the small sample size.

Expression of MAGE-A and NY-ESO-1 in Primary and Metastatic Squamous Cell Cancer

A total of 38 patients with primary or metastatic squamous cell cancers from various origins were examined for the expression of MAGE-A and NY-ESO-1 (Table 5). Overall we found that 9 of 20 (45%) of metastatic lesions had MAGE positivity and 8 of 18 (44%) of primary lesions had MAGE positivity. There was much less NY-ESO-1 positivity with 3 of 38 (8%) positivity overall, with 3 of 20 (15%) metastatic lesions expressing NY-ESO-1 and 0 of 18

primary lesions. Supplementary Figure A (Supplemental Digital Content 1, <http://links.lww.com/JIT/A405>) shows the organ of origin from which expression was determined.

Expression of MAGE-A and NY-ESO-1 in Primary and Metastatic Adenocarcinomas

A total of 111 patients seen in the Surgery Branch, National Cancer Institute with primary or metastatic adenocarcinomas of different origins were examined for expression of MAGE-A and NY-ESO-1 (Table 6). Overall we found 10% of patients expressed MAGE-A, and 1% expressed NY-ESO-1. In 25 breast cancer patients, 1 of 13 (8%) of primary and 2 of 12 (17%) of metastatic lesions expressed MAGE-A positivity. Of cancers of GI origin, 7 of 14 (14%) expressed MAGE positivity. Three of 25 (12%) of primary and 4 of 24 (17%) metastatic GI lesions were positive for MAGE. Of note none of the 23 pancreatic cancer lesions, both primary and metastatic, were positive for MAGE. Two of 5 (40%) of urothelial carcinomas expressed MAGE. Lung, cervical, and ovarian histologies were negative but the sample size was small. NY-ESO-1 positivity was only seen in 1 of 25 breast cancer lesions, and all other histologies were negative for NY-ESO-1 positivity.

DISCUSSION

CTAs are ideal targets for cancer immunotherapies due to their restricted expression in various malignancies and germline cells. As germ cells do not express MHC class I molecules, they are immune privileged.² CTAs are expressed in a variety of human malignancies including melanoma and carcinomas of bladder, lung, liver, breast, ovary, and synovial cells.^{3–13} In addition, T-cell receptor-based immune-therapies targeting NY-ESO-1 have been shown to result in durable clinical responses in both metastatic melanoma and synovial cell cancer patients.^{15,16,18} T-cell receptor therapies targeting MAGE-A3 have shown tumor regression in advanced melanoma patients.¹⁵

Here we have assessed the expression of MAGE-A and NY-ESO-1 in both primary and metastatic lesions from 301 melanoma, 38 squamous cell cancer and 111 adenocarcinoma patients evaluated for protocols at the NIH surgery branch. Patient selection for treatment was determined in part by percentage and intensity of expression of these CTAs. For the goals of patient selection into these protocols, it would be important to determine the level of expression in both primary and metastatic lesions throughout multiple histologies and consistency of expression in multiple metastatic sites per patient. We also analyzed the concordance between multiple metastatic lesions per patient in 47 metastatic melanoma patients and relative levels of expression of MAGE-A and NY-ESO-1 between paired primary and metastatic lesions within 13 individual melanoma patients.

For melanomas, MAGE-A is more highly expressed than NY-ESO-1 (32% vs. 13%). Both MAGE-A and NYESO are more highly expressed in metastatic lesions versus primary lesions in melanoma patients.

Our study showed NY-ESO-1 positivity in 38 of 274 (14%) of metastatic tumors and 2 of 40 (5%) primary tumors. Prior studies report higher levels of positivity. Aung and colleagues, demonstrated NY-ESO-1 positivity was found in 58 of 206 (28.2%) metastatic tumors and 0 of 16 (0%) primary tumors. In another study, 18 of 36 (32%) metastatic and 8 of 61 (13%)

primary melanomas were positive for NY-ESO-1 IHC expression.⁵ Vaughan et al¹⁹ compared NY-ESO-1 reverse transcription-polymerase chain reaction (RT-PCR) with IHC and found for 120 metastatic melanoma lesions that 48 of 120 (40%) were positive by RT-PCR and 54 of 120 (45%) were positive by IHC. Similarly Bolli et al²⁰ found that by IHC 12 of 38 (32%) of melanoma lesions were positive for NYESO-1.

Notably, the discrepancy over the level of NY-ESO-1 staining in our study compared with the other studies is likely due to the fact that our criteria for positivity was 50%, whereas their cutoff ranged from 1% to 10%. This study is the first to assess MAGE expression by IHC in a large number of melanomas, MAGE expression in melanoma has been reported only by RT-PCR data ranging from 53% to 74% in melanomas by RT-PCR.^{2,21}

In our study, within paired primary and metastatic samples, MAGE-A expression is higher in metastatic lesions in 5 of 13 patients, and no patient expressed higher levels in their primary tumor compared with their metastatic lesion. For NY-ESO-1, the paired lesions were mostly both negative (11/13) and so similar conclusions could not be drawn. These data would support MAGE-A targeted immunotherapy in metastatic patients rather than early stage patients. Aung and colleagues found that in 9 paired samples, 6 were negative for NY-ESO-1 in both primary and metastatic lesions, but the remaining 3 showed positive staining in the metastatic site and no staining in the primary site. This difference may be due to our criteria of positivity, as well as small sample size.^{5,8}

It is also of importance to assess concordance of CTA expression in multiple metastatic lesions within the same patient. We found that only 9 of 47 (20%) patients had discordant expression of MAGE-A and 4 of 47 (8%) for NY-ESO-1 expression among their metastatic lesions.

For squamous cell cancers, we assessed 38 patients including 18 primary and 20 metastatic lesions. MAGE-A was more highly expressed than NY-ESO-1 (45% vs. 7.9%). There was similar expression of MAGE-A between primary and metastatic groups (45%) and higher expression of NY-ESO-1 in metastatic lesions (15%) versus primary lesions (0%).

Other studies have shown similar levels of expression of MAGE-A by IHC including, 40% expression in 453 patients with primary SCC of the head and neck¹² and 30 of 55 (54%) of primary esophageal SCC cancers and 12 of 28 (42%) in metastatic lesions.¹⁰

RNA expression ranged from 21.4% to 51% in previous studies evaluating MAGE-A in SCC. MAGE-A positivity was found in 51% of a panel of 57 primary head and neck squamous cell cancer.²² Eleven of 37 (29%) of primary cervical cell cancers were found to be positive for MAGE RNA expression.²³ Six of 28 (21.4%) primary esophageal SCCs were found to be positive for MAGE by RT-PCR.²⁴

NY-ESO-1 expression in other studies have been reported to be higher by RNA including 41 of 123 (33%) esophageal SCCs, from which 87% of RNA + samples were found to be also positive by IHC.²⁵ Other studies report NY-ESO-1 positivity with 11 of 46 (23%) by RNA and 18 of 56 (32%) by IHC in esophageal SCCs.^{7,26} NY-ESO was positive in 19 of 55

(32.7%) and 5 of 28 (17.9%) in primary and metastatic lesions, respectively, by IHC in esophageal SCCs.¹⁰

For adenocarcinomas, MAGE-A and NY-ESO-1 expression was much less than compared with melanomas or squamous cell cancers with an overall expression of 10% and 0.9%, respectively. The most expression in breast, GI, and urothelial carcinomas were for MAGE-A3 and only 1 breast cancer patient had NY-ESO-1 positivity.

MAGE expression by RNA was reported in 24 of 74 (32%) bladder cancers.²⁷ IHC positivity is reported to be 290 of 480 (60%) of primary tumors and 28 of 33 (85%) of lymph node metastases by IHC for MAGE-A9.⁹

NY-ESO-1 was found to be positive in a set of studies on bladder transitional cell carcinoma by RNA expression in 15 of 43 (35%) and 20 of 62 (32%). Positivity by IHC for NY-ESO-1 was seen in 22 of 72 (31%), 2 of 14 (14%), and 6 of 33 (18%).^{4,20,28} Renal cell cancers demonstrated MAGE-A3 RNA positivity in 27.6% and NY-ESO in 21%.²⁹

MAGE IHC expression in breast cancers are reported to be 36 of 82 (43%),³⁰ RNA expression for MAGE-A9 and MAGE-A11 were 94 of 210 (45%) and 140 of 210 (66.7%), respectively.³¹ NY-ESO-1 positivity by RT-PCR was found to be 18 of 67 (27%), 37 of 88 (42%), and 13 of 129 (10%) of primary breast cancers.^{26,32,33}

Odunsi and colleagues found that in epithelial ovarian cancers, NY-ESO was positive by RT-PCR in 32 of 107 (30%) and 21 of 142 (14.7%) by IHC. In lung adenocarcinomas, 6 of 33 (18%) were positive by both RT-PCR and IHC for NY-ESO-1.¹¹

Colorectal cancers had MAGE expression by RNA in 23 of 82 (28%) and 19 of 37 (51%),^{3,34} and 35 of 89 (39%) by IHC.³⁵ Low NY-ESO RNA expression in 2 of 98 (2%), 12 of 121 (10%) colon cancer patients, and 0 of 61 (0%) pancreatic cancer patients were reported.^{26,36,37}

Our findings for MAGE-A and NY-ESO-1 in breast (M, 12%; E, 4%), GI (M, 14%; E, 0%), and urothelial cancers (M, 40%; E, 0%) are lower than reported in the literature but our sample size per cancer type was small and our criteria for positivity is much higher.

In conclusion, we have found that MAGE-A is more highly expressed by IHC than NY-ESO-1 in metastatic and primary melanomas, squamous cell cancers and adenocarcinomas. Both MAGE-A and NY-ESO-1 are more highly expressed in metastatic lesions versus primary lesions in melanoma patients and which suggests that immunotherapies that target these antigens may be more efficacious in the metastatic setting. In addition, there was only 19% discordance between multiple metastatic lesions per patient, whereas the remaining 81% had consistent levels of IHC expression in their metastatic lesions. MAGE-A was highly expressed in a cohort of squamous cell cancer patients compared with NY-ESO-1 and may be a more valuable immunotherapeutic target in this histology. MAGE-A was also more highly expressed in adenocarcinomas compared with NY-ESO-1, but overall much less when compared with melanomas and squamous cell cancers. However, our sample size per cancer type in the adenocarcinomas was small.

Our findings may suggest that targeted immunotherapy against MAGE-A is likely to be more valuable than NY-ESO-1 particularly in melanomas and squamous cell carcinomas.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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CONFLICTS OF INTEREST/FINANCIAL DISCLOSURES

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FIGURE 1.

Representative examples of melanoma-associated antigen-A (MAGE-A) and New York esophageal squamous cell cancer-1 (NY-ESO-1) expression in primary and metastatic melanomas. A, NY-ESO-1 expression, 1 + intensity. B, NY-ESO-1 expression, 2 + intensity. C, NY-ESO-1 Expression, 3+ intensity. D, MAGE-A expression, 1 + intensity. E, MAGE-A expression, 2+ intensity. F, MAGE-A expression, 3 + intensity.

TABLE 1.

Expression of MAGE-A and NY-ESO-1 in Primary and Metastatic Melanoma

	MAGE-A			NY-ESO-1		
	Percentage	1-1 +	2-3 +	Intensity	1-1 +	2-3 +
Primary lesions (n = 40)						
Negative						
			32			37
1%–49%		3	2			1
50%–74%			2			2
75%–100%		1	1			
> 50%, 1-2-3 +		3/40	7.5%			5.0%
				Negative		
				1%–49%		
				50%–74%		
				75%–100%		
				> 50%, 1-2-3 +		
					2/40	
Metastatic lesions (n = 274)						
			116			96
Negative						
		26	34			40
1%–49%					14	26
50%–74%		2	43		6	15
75%–100%		2	51		4	13
> 50%, 1-2-3 +		98/274	36%		38/274	14%
				Negative		
				1%–49%		
				50%–74%		
				75%–100%		
				> 50%, 1-2-3 +		
					40/314	13%
All lesions (n = 314)						
			148			96
Negative						
		29	36			41
1%–49%					14	27
50%–74%		2	45		7	16
75%–100%		2	52		4	13
> 50%, 1-2-3 +		101/314	32%		40/314	13%
				Negative		
				1%–49%		
				50%–74%		
				75%–100%		
				> 50%, 1-2-3 +		
					40/314	13%

MAGE-A indicates melanoma-associated antigen-A; NY-ESO-1, New York esophageal squamous cell cancer-1.

TABLE 2.

Expression of MAGE-A and NY-ESO in Melanoma Patients With Multiple Metastatic Lesions

	MAGE-A	NY-ESO-1
All negative	20	41
Mixed negative/positive (discordant)	9	4
All positive	18	2
Total patients	47	47

MAGE-A indicates melanoma-associated antigen-A; NY-ESO-1, New York esophageal squamous cell cancer-1.

TABLE 3.

MAGE-A and NY-ESO-1 Expression in Melanoma Patients With Paired Primary and Metastatic Lesions (n = 13)

Primary	Metastatic	MAGE-A	NY-ESO-1
Negative	Negative	8	11
Positive	Negative	0	0
Negative	Positive	5	1
Positive	Positive	0	1
Total patients		13	13

MAGE-A indicates melanoma-associated antigen-A; NY-ESO-1, New York esophageal squamous cell cancer-1.

TABLE 4.
Expression of MAGE-A and NY-ESO-1 in Primary and Metastatic Melanomas From Various Organ Sites

	MAGE-A			NY-ESO-1		
	Positive	Negative	Total	Positive	Negative	Total
Primary						
Skin	3	35	38	2	36	38
Rectum/anus	0	2	2	0	2	2
Metastatic						
Adrenal	2	1	3	1	2	3
Bone	2	2	4	1	3	4
Breast	1	2	3	1	2	3
Brain	4	0	4	1	3	4
GI	11	4	15	4	11	15
GU	1	1	2	0	2	2
Kidney	0	2	2	0	2	2
Liver	4	28	32	5	27	32
Lymph node	23	57	80	5	75	80
Lung	6	20	26	5	21	26
Muscle	2	1	3	0	3	3
Salivary	0	2	2	0	2	2
Skin	17	25	42	6	36	42
Spleen	1	2	3	0	3	3
Soft tissue	22	31	53	9	44	53
	99	215	314	40	274	314

MAGE-A indicates melanoma-associated antigen-A; NY-ESO-1, New York esophageal squamous cell cancer-1.

TABLE 5.
Expression of MAGE-A and NY-ESO-1 in Primary and Metastatic Squamous Cell Cancers

	MAGE-A			NY-ESO-1		
	Percentage	1-1 +	2-3 +	Percentage	1-1 +	2-3 +
Primary Lesions (n = 18)						
Negative			7	Negative		18
1%-49%		1	1	1%-49%		
50%-74%		1	3	50%-74%		
75%-100%			4	75%-100%		
> 50%, 1-2-3 +			8/18	> 50%, 1-2-3 +	0/18	0.0%
Metastatic lesions (n = 20)						
Negative			11	Negative		17
1%-49%		1		1%-49%		
50%-74%			4	50%-74%	1	1
75%-100%			5	75%-100%	1	1
> 50%, 1-2-3 +			9/20	> 50%, 1-2-3 +	3/20	15.0%
All lesions (n = 38)						
Negative			18	Negative		36
1%-49%		2	1	1%-49%		
50%-74%		1	7	50%-74%	1	1
75%-100%			9	75%-100%	1	1
> 50%, 1-2-3 +			17/38	> 50%, 1-2-3 +	3/38	7.9%

Positive staining defined as 50%, 1-3 +.

MAGE-A indicates melanoma-associated antigen-A; NY-ESO-1, New York esophageal squamous cell cancer-1.

TABLE 6.

Expression of MAGE-A and NY-ESO-1 in Primary and Metastatic Adenocarcinomas

Histology	MAGE-A			NY-ESO-1		
	Primary	Metastatic	Total	Primary	Metastatic	Total
Breast	1/13 (8%)	2/12 (17%)	3/25 (12%)	0/13	1/12 (8%)	1/25 (4%)
GI	3/25 (12%)	4/24 (17%)	7/49 (14%)	0/24	0/21	0/49
Cervical	0/1	0/2	0/3	0/1	0/2	0/3
Ovarian	0/2	0/2	0/4	0/2	0/2	0/4
Lung	0/1	0/1	0/2	0/1	0/1	0/2
Urothelial	1/2 (50%)	1/3 (33%)	2/5 (40%)	0/2	0/3	0/5
Pancreas	0/12	0/11	0/23	0/12	0/11	0/23
Total			12/111 (10%)			1/111 (1%)

MAGE-A indicates melanoma-associated antigen-A; NY-ESO-1, New York esophageal squamous cell cancer-1.