

Antibody Immunity to the p53 Oncogenic Protein Is a Prognostic Indicator in Ovarian Cancer

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A B S T R A C T

Purpose

Presence of intratumoral T-cell infiltration has been linked to improved survival in ovarian cancer patients. We questioned whether antibody immunity specific for ovarian cancer tumor antigens would predict disease outcome. We evaluated humoral immune responses against ovarian cancer antigens p53, HER-2/*neu*, and topoisomerase II α .

Patients and Methods

Serum was collected from 104 women (median age, 59 years; range, 34 to 89 years) at the time of their initial definitive surgery for ovarian cancer. Serum was analyzed by enzyme-linked immunosorbent assay for antibodies to p53, HER-2/*neu*, and topoisomerase II α proteins. Antibody immunity to tetanus toxoid was assessed as a control. The incidence of humoral immunity at the time of diagnosis to any of these three antigens was tabulated. For patients with advanced-stage disease (III/IV), correlation was made between the presence of tumor-specific immunity at the time of diagnosis and overall survival. Patients were followed for a median of 1.8 years.

Results

Multivariate analysis showed the presence of p53 antibodies to be an independent variable for prediction of overall survival in advanced-stage patients. Overall survival was significantly higher for patients with antibodies to p53 when compared with patients without p53 antibodies ($P = .01$). The median survival for p53 antibody-positive patients was 51 months (95% CI, 23.5 to 60.5 months) compared with 24 months (95% CI, 19.4 to 28.6 months) for patients without antibodies to p53.

Conclusion

Data presented here demonstrate that advanced stage ovarian cancer patients can have detectable tumor-specific antibody immunity and that immunity to p53 may predict improved overall survival in patients with advanced-stage disease.

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INTRODUCTION

Ovarian cancer can elicit **immunity** in patients with the disease. Several tumor antigens have been identified in women with ovarian cancer. These **immunogenic** proteins stimulate a specific immune response in individuals with ovarian cancer but not in non-tumor bearing individuals.¹ Recently, a large retrospective study demonstrated that the presence of intratumoral T-cell infiltrates in ovarian tumors predicted both improved progression-free and overall survival in women with advanced-stage disease regardless of response to treatment.² This study was a significant demonstration that tumor-directed immunity may have an impact on clinical outcome in ovarian cancer.

Proteins that are overexpressed by tumor cells or that accumulate in tumor cells are more readily

available for immune recognition than the same proteins expressed at basal levels in noncancerous cells.³ The level of HER-2/*neu* overexpression in primary tumor is associated with production of HER-2/*neu*-specific antibodies, and there is a strong correlation between accumulation of p53 in primary tumor cells and presence of serum p53-specific antibodies in patients with different cancers.⁴⁻⁶ Moreover, antigen-specific antibody immunity is positively associated with antigen-specific T-cell responses, indicating that **immunoglobulin G** (IgG) immunity may act as a marker for the presence of CD4⁺ and CD8⁺ T-cell immunity.^{1,7,8}

We developed assays to detect **humoral** immunity against a panel of known ovarian cancer antigens that are overexpressed or abundant in tumor cells (p53, HER-2/*neu*, and topoisomerase II α). More importantly, these proteins are known to be implicated in the pathogenesis of the disease.

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Terms in **blue** are defined in the glossary, found at the end of this article and online at www.jco.org.

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Expression of p53, HER-2/*neu*, or topoisomerase II α in ovarian tumors is associated with more aggressive disease and a decreased survival.⁹⁻¹² For this reason, we hypothesized that detectable immunity against these particular proteins may be associated with a survival benefit.

Serum was collected prospectively from 104 women before primary surgery for ovarian cancer. The patients were then followed for a median of 1.8 years, and survival data were collected. Antibody immunity to p53 at the time of diagnosis predicted improved survival among patients with advanced-stage (III/IV) disease. The survival benefit was specific for immunity to the tumor-related protein and not a reflection of general immune competence as evidenced by lack of survival benefit associated with humoral immunity to tetanus toxoid (TT), a control antigen.

PATIENTS AND METHODS

Patient Samples

Serum samples were collected via the ORCHID study (http://www.fhrc.org/research/diseases/ovarian_cancer/#overview), an institution review board-approved biomarker trial, conducted at the Fred Hutchinson Cancer Research Center (Seattle, WA). A total of 256 women were recruited from 1998 to 2001. Women between the ages of 18 and 75 years scheduled for complete abdominal hysterectomy or oophorectomy, with or without suspected malignancy, were eligible for participation. Blood samples were drawn after written consent at time of surgery, before removal of the ovaries. One hundred eighteen of the 256 women were subsequently diagnosed with ovarian malignancy, and selected clinical data were collected on those patients during the time of their treatment and follow-up. The clinical data collected were limited to age, stage of disease, histology, and CA-125 level at time of diagnosis. Patient demographics are presented in Table 1. For the study reported here, only patients with complete histologic, staging, and survival data were analyzed. Histology data were not available for two patients, staging data for four patients, and longitudinal follow-up for eight patients. Thus, 104 samples were available for study. The coded samples were analyzed as described in the section Enzyme-Linked Immunosorbent Assay Analysis for Antibody Immunity to Oncogenic Proteins, and once biomarker data were finalized, clinical information was linked, and analysis was performed as described. Limited disease was defined as stage I/II, and advanced disease was defined as stage

III/IV disease. Overall survival was defined as the time elapsed between beginning initial treatment (surgical or chemotherapeutic) and death. Data for patients without death were censored at time of last contact. The median time of longitudinal follow-up since the initial collection of the samples at the time of this analysis is 22 months (range, 0 to 75 months). Fifty-five deaths were observed in this cohort of 104 women. Survival analysis included only advanced-stage (III/IV) patients (n = 80), because they are the majority of patients, and time to follow-up is sufficient to evaluate outcome.

Control Population

Serum from 175 female donors between ages 18 and 75 years was used to establish a reference interval for each marker. Serum samples were obtained from the Puget Sound Blood Bank in Seattle, WA, and the volunteers met all criteria for blood donation. Control sera were also stored at -80°C.

Enzyme-Linked Immunosorbent Assay Analysis for Antibody Immunity to Oncogenic Proteins

Antibodies to p53 and HER-2/*neu* were evaluated using a cell-based assay.¹³ For analysis of HER-2/*neu* antibody immunity, 96-well microtiter plates (Dynex Technologies, Inc, Chantilly, VA) were coated with 520C9, a monoclonal antibody specific to HER-2/*neu*, and diluted to a concentration of 10 μ g/mL with carbonate buffer. Serially diluted, purified human IgG (Sigma) provided a standard curve. Plates were incubated overnight at 4°C. All wells were then blocked with 100 μ L/well of a filtered buffer of 10% phosphate-buffered saline (PBS)/1% bovine serum antigen (BSA; Sigma Chemical Co, St Louis, MO), 100 μ L/well, and incubated at room temperature on a rocker for 4 hours. Plates were washed four times with 10% PBS/0.5% Tween (Amersham Biosciences, Piscataway, NJ) before addition of SKBR3, a cell line used as a source of HER-2/*neu* protein, diluted with PBS/BSA buffer to 20 μ g/mL at 50 μ L/well. Plates were again incubated overnight at 4°C. Plates were then washed four times with PBS/Tween, and patient sera were added to wells. Serum from a patient with HER-2/*neu* overexpressing breast cancer was used as a positive control. The positive control was run on every plate, as was a negative control of PBS/BSA buffer treated as a serum sample. After serum incubation, plates were washed four times with PBS/Tween and goat antihuman IgG-horseradish peroxidase (HRP) conjugate (Zymed Laboratories, South San Francisco, CA) added at a dilution of 1:50,000 (50 μ L/well) and incubated for 45 minutes at room temperature on rocker. After a final PBS/Tween wash, developing reagent was added (75 μ L/well) and color reaction assessed at an optical density (OD) of 640 nm until the well containing the standard at a concentration of 0.16 μ g/mL evaluated at 0.3 OD. Reaction was then stopped with 75 μ L/well 1N HCL and read at OD of 450 nm. The OD of each serum dilution was calculated as the OD of the lysate-coated wells minus the OD of the

Table 1. Patient Characteristics

Stage	No.	CA-125 (U)		Age (years)		Histology	
		Median	Range	Median	Range	Type	No.
I	20	94	0-482	58	41-89	Serous	10
						Mucinous	7
						Endometrioid	2
						Other*	1
II	4	1,177	20-4,491	45	34-67	Serous	2
						Other*	2
III	67	1,289	0-14,842	62	37-84	Serous	58
						Undifferentiated	5
						Clear cell	1
						Other*	3
IV	13	955	62-3,681	64	41-86	Serous	9
						Endometrioid	2
						Other*	2
Total							104

*Malignant adenosarcoma, mullarian mixed tumor, clear cell carcinoma, adenocarcinoma not otherwise specified, or unclassified epithelial tumor.

PBS/BSA-coated wells. Values for each Δ OD were calculated from the log-log equation of the line for the standard curve on each plate, as plotted by SOFTmax version 2.3 for Macintosh (Molecular Devices Corp, Sunnyvale, CA). A sample was defined as positive if the value was greater than the mean and two standard deviations of the previously analyzed reference population. The anti-p53 enzyme-linked immunosorbent assay (ELISA) was performed similarly as that described in this paragraph, with the following modifications: Plates were coated with TIB116, a murine monoclonal antibody specific for human p53 (American Type Culture Collection [Manassas, VA] #TIB-116), and the BT-20 cell line was used as a source of p53 protein.

Antibodies to topoisomerase II α and TT were assessed using an indirect ELISA method with recombinant proteins. Briefly, alternate columns on Immulon 4HBX plates (Dynex Technologies, Chantilly, VA) were coated overnight with purified human topoisomerase II α (Topogen, Columbus, OH) or TT antigen (Sigma Chemicals Inc, St. Louis, MO) and PBS/BSA, blocked for 1 hour with PBS/BSA, and washed with PBS/Tween. After washing, 50 μ L/well of control or experimental sera were added in duplicate titration sets. After overnight incubation at 4°C, plates were washed again and antihuman/HRP conjugate added 50 μ L/well. Plates were washed again after a 45-minute incubation at 4°C, and developed as previously described.^{13,14} Seventy-five of 104 samples had sufficient remaining volume to test for immunity to topoisomerase II α .

A positive sample was defined as an antibody concentration above the nonparametric 95th percentile of the control samples evaluated for each antigen. For p53 antibodies, this value was $0.16 \pm 0.25 \mu\text{g/mL}$; $0.15 \pm 0.49 \mu\text{g/mL}$ for HER-2/*neu* antibodies; and $0.1 \pm 0.27 \mu\text{g/mL}$ for topoisomerase II α antibodies. All assays were linear over a range (0.001 to 11.0 $\mu\text{g/mL}$) of antibody concentrations tested with a correlation coefficient of $r = 0.999$ for the p53 assay, $r = 0.997$ for HER-2/*neu*, and $r = 0.995$ for topoisomerase II α . On the basis of repeated measures of 10 control and 10 experimental sera over a 6-month period, the intra- and interassay coefficient of variations for the p53 ELISA were 12% and 15%, respectively, 9% and 20% for HER-2/*neu* were, and 10% and 14% for topoisomerase II α . Positive responses were verified by Western blot analysis.¹⁵ The sensitivity and specificity were 93% and 100% for p53, 89% and 77% for HER2, and 83% and 100% for the topoisomerase II α .

Statistical Analysis

Significance of proportional differences was quantified by Fisher's exact test. Two-way comparisons of antibody levels between patients with advanced disease and limited disease, or between ovarian cancer patients and volunteer controls, were analyzed by Mann-Whitney *U* test. Kaplan-Meier curves were constructed and log-rank analysis was used to make univariate comparisons between antibody-positive and antibody-negative patients with advanced-stage cancer. Multivariate analysis of overall survival in patients with advanced-stage disease was performed using the Cox proportional hazards regression method. Stage (III or IV) and presence of p53, HER-2/*neu*, or topoisomerase II α antibodies were included in the model as binary variables, CA 125 level at time of diagnosis and age as continuous variables. All analysis was by Statistical Package for the Social Sciences version 13.0 (SPSS Inc, Chicago, IL).

RESULTS

Antibody Immunity to Oncogenic Proteins Is Associated With Advanced-Stage Ovarian Cancer

Sera from women with advanced-stage (III/IV) disease were more likely to have detectable antibodies specific for p53 ($n = 104$), HER-2/*neu* ($n = 104$), or topoisomerase II α ($n = 75$) than samples from patients with limited stage (I/II) disease (Fig 1). The percentage of patients demonstrating p53 antibody immunity increased significantly ($P = .006$), from 6% in limited stage patients to 30% in advanced stage. Nineteen percent of samples from women with limited-stage disease contained detectable HER-2/*neu*-specific antibodies as compared with 22% from advanced-stage patients. The

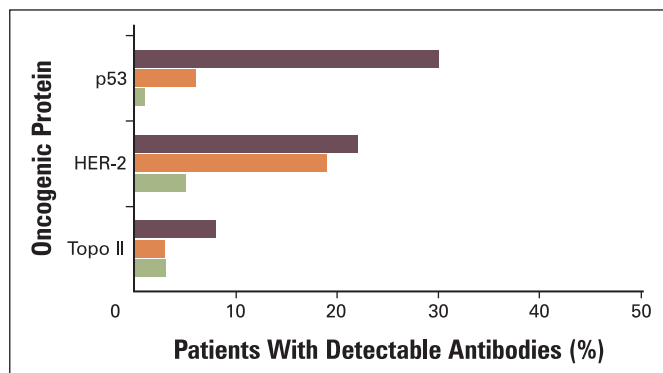


Fig 1. Proportion of samples positive for antibodies is greater in advanced disease than in limited disease. Percentage of samples positive for antibodies to p53, HER-2/*neu* (HER-2), or topoisomerase II α (Topo II) is shown for advanced disease (purple), limited disease (orange) ovarian cancer patients, and volunteer controls (green).

percentage positive for topoisomerase II α increased from 3% in limited-stage to 8% in advanced-stage disease. The increased presence of antibodies to HER-2/*neu* and topoisomerase II α in women with advanced-stage ovarian cancer was not statistically significant as compared with more limited-stage disease.

The mean level of p53-specific IgG for the advanced-stage patients was 3.6 $\mu\text{g/mL}$ (range, 0 to 56.6 $\mu\text{g/mL}$) and for the limited-stage group was 0.12 $\mu\text{g/mL}$ (range, 0 to 1.0 $\mu\text{g/mL}$). The difference in magnitude of p53 antibody immunity was statistically different between these two groups ($P = .02$). The mean level of HER-2/*neu*-specific IgG for the advanced-stage group was 0.61 $\mu\text{g/mL}$ (range, 0 to 9.2 $\mu\text{g/mL}$), and for the limited-stage group was 0.5 $\mu\text{g/mL}$ (range, 0 to 3.3 $\mu\text{g/mL}$), not a statistically significant difference ($P = .93$). The mean topoisomerase II α IgG response for the advanced-stage patients was 0.2 $\mu\text{g/mL}$ (range, 0 to 2.6 $\mu\text{g/mL}$) and for the limited-stage women was 0.10 $\mu\text{g/mL}$ (range, 0 to 1.1 $\mu\text{g/mL}$), not a statistically significant difference ($P = .90$). In addition, antibody immunity to oncogenic proteins readily distinguished patients with cancer from controls (p53, $P = .0001$; HER-2/*neu*, $P = .002$; and topoisomerase II α , $P = .017$). The mean of healthy controls was 0.16 $\mu\text{g/mL}$ (range, 0 to 1.7 $\mu\text{g/mL}$) for the p53 assay, 0.15 $\mu\text{g/mL}$ (range, 0 to 3.7 $\mu\text{g/mL}$) for the HER-2/*neu* assay, and 0.1 $\mu\text{g/mL}$ (range, 0 to 2.0 $\mu\text{g/mL}$) for the topoisomerase II α assay (Fig 2).

Antibody Immunity to p53 in Women With Advanced-Stage Ovarian Cancer Is Associated With Increased Survival As Compared With Women Without p53 Antibody Immunity

Kaplan-Meier curves comparing overall survival between women with p53 antibody-positive and antibody-negative advanced-stage ovarian cancer indicate that overall survival is increased in those women with immunity (Fig 3). The median survival for p53 antibody-positive patients was 51 months (range, 23.5 to 60.5 months) compared with 24 months (95% CI, 19.4 to 28.6 months) for patients without antibodies to p53 (log-rank $P = .01$; Fig 3A). There was no survival benefit related to humoral immunity to the other oncogenic proteins evaluated. The median overall survival for women with antibody immunity to HER-2/*neu* was 42 months (95% CI, 6.7 to 77.3) compared with 27 months (95% CI, 18.2 to 35.8; $P = .32$), and the median overall survival in women with topoisomerase II α antibodies

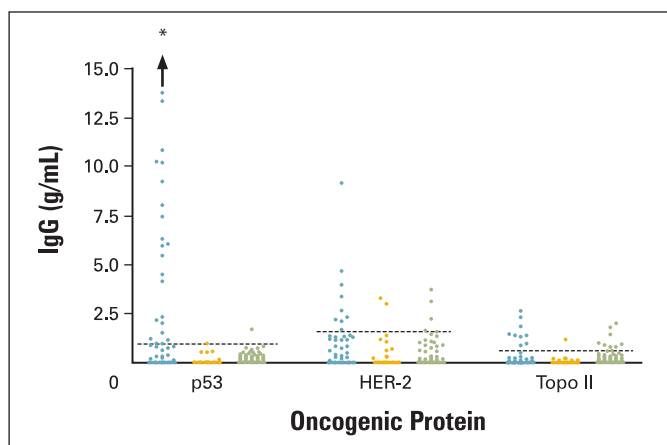


Fig 2. Antibody response is higher in advanced disease. Level of antibody response to p53, HER-2/neu[r] (HER-2), or topoisomerase II α (Topo II) is shown for advanced disease (blue), limited disease (gold) patients, and controls (green). Dotted lines represent positive cut points. (*) denotes three values off the graph; 43.2 μ g/mL, 53.7 μ g/mL, 56.6 μ g/mL.

was 15 months (95% CI, 0 to 33.0) compared with those women without antibodies, whose survival was 36 months (95% CI, 21.1 to 50.9; $P = .29$; Fig 3B and C). Multivariate analysis showed that the presence of p53 antibodies was an independent variable for prediction of overall survival in advanced-stage patients. Overall survival was significantly higher for patients with antibodies to p53 when compared with patients without p53 antibodies (hazard ratio, 2.46; 95% CI, 1.52 to 4.18; $P = .01$). In this study population, stage, age, and CA-125 levels at the time of diagnosis were not significant predictors for overall survival ($P = .98$, $P = .07$, and $P = .17$ respectively), and neither was the presence of HER2 antibodies ($P = .45$), nor the presence of topoisomerase II α antibodies ($P = .28$).

Improved Survival Associated With Antibody Immunity to Oncogenic Proteins, in Women With Advanced-Stage Ovarian Cancer, Is Not a Reflection of General Immune Competence

A possible explanation for the potential of antibody immunity to oncogenic proteins to predict survival in women with advanced-stage ovarian cancer is that the ability to mount an immune response is a predictor of good performance status. To address this question, we assessed for the presence of TT-specific antibodies in the women with advanced-stage ovarian cancer as an evaluation of immune competence. The median overall survival was 45 months (95% CI, 19.4 to 51.2) for women who had antibodies to TT and 30 months (95% CI, 12.8 to 41.4) for tetanus antibody-negative patients (Fig 4). These differences were not significant ($P = .34$). In addition, the disease characteristics were similar between antibody-positive and antibody-negative patients, stage ($P = .09$), age ($P = .37$), and CA-125 level at the time of diagnosis ($P = .87$). The development of antibody immunity to multiple tumor-associated proteins may indicate a significant trend toward increased survival ($P = .05$), with a median overall survival time of 24 months for patients negative for any marker, 38 months for patients positive for any one marker, and 42 months median overall survival time for patients positive for any two markers.

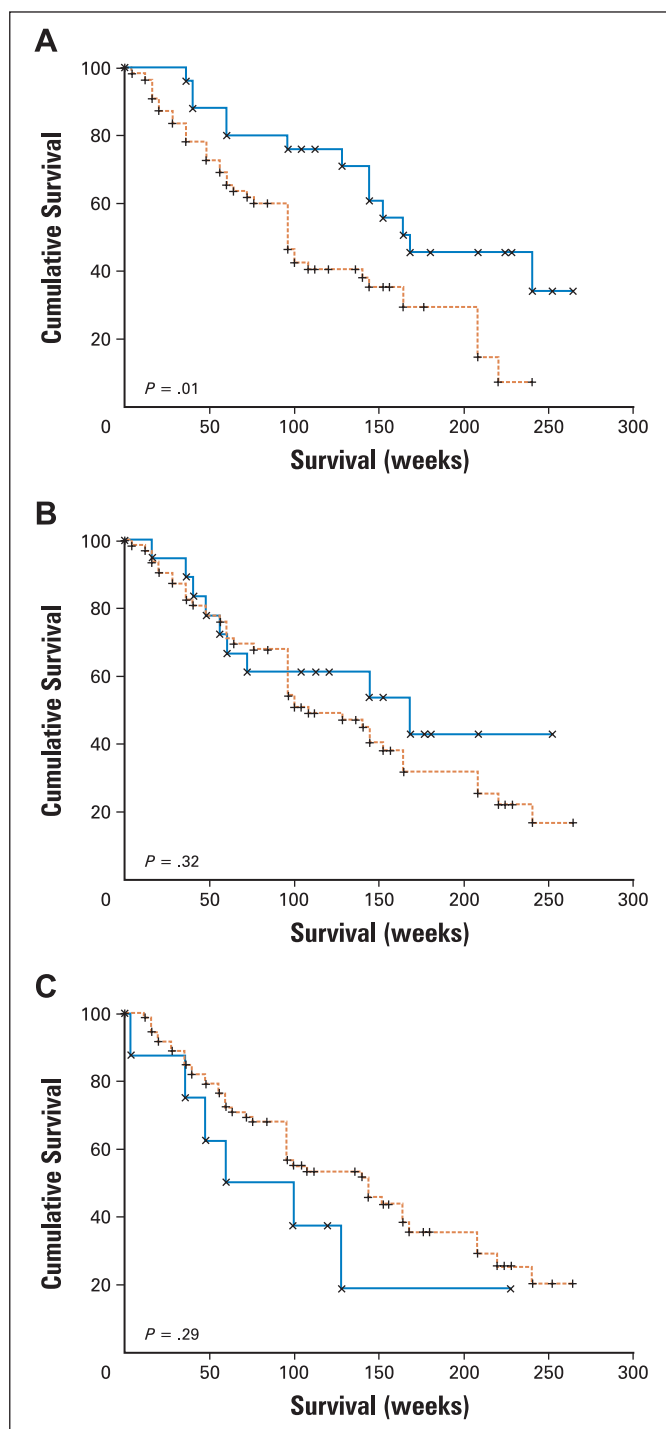


Fig 3. Overall survival is significantly increased in advanced stage ovarian cancer patients with antibodies to p53. Kaplan-Meier curves comparing overall survival between (A) p53 antibody-positive (blue) and antibody-negative (red) patients; (B) HER-2/neu[r] antibody-positive (blue) and antibody-negative (red) patients; and (C) topoisomerase II α antibody-positive (blue) and antibody-negative (red) patients.

DISCUSSION

We evaluated IgG-specific antibody immunity to p53, HER-2/neu, and topoisomerase II α , all immunogenic proteins in ovarian cancer and implicated in the pathogenesis of the disease. IgG antibody

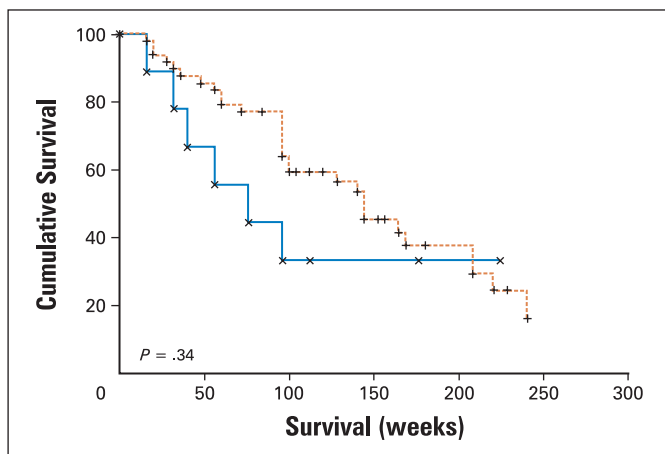


Fig 4. There are no differences in overall survival between patients positive for tetanus toxoid antibodies and patients negative for tetanus toxoid antibodies. Kaplan-Meier curves comparing overall survival between patients positive for tetanus toxoid antibodies (blue) and negative for tetanus toxoid antibodies (red).

immunity has been shown to be predictive of a concomitant T-cell response, because Ig class switching from IgM to IgG requires cognate T-cell help.^{15,16} Thus, antigen-specific IgG antibody immunity may be a predictor of the presence of antigen-specific T cells. Data presented herein demonstrate that women with advanced-stage ovarian cancer have tumor-specific antibody immunity and that immunity to p53 predicts improved overall survival in patients with advanced-stage ovarian cancer.

A concern of evaluating immunity in women with ovarian cancer is that the majority of patients are diagnosed at an advanced stage. Factors involved in ovarian cancer progression, such as secretion of immunosuppressive cytokines,¹⁷ elaboration of T-regulatory cells,¹⁸ and increasing infiltration of monocytes that dampen the development of T-cell immunity,¹⁹ can prevent immunity from developing. One assumption might be that the tumor-specific humoral immune response would be more predominant in individuals with limited- or early-stage disease. Data presented here demonstrated that antibody immunity was present in women with advanced-stage ovarian cancer; indeed, more than half of the women studied had some detectable antibody immunity against at least one of the tumor antigens evaluated. Investigations by Zhang et al² indicated that more than half of women with advanced-stage ovarian cancer had evidence of intra-tumoral T cells. Such data suggest that although a variety of immunosuppressive mechanisms are associated with the ovarian cancer microenvironment, patients still have the potential to mount an immune response against their tumor.

We developed assays to assess humoral immunity to three tumor antigens expressed in ovarian cancer: p53, HER-2/*neu*, and topoisomerase II α . All three of these proteins have been shown to be immunogenic in individuals with ovarian cancer.²⁰⁻²² In addition, expression of these proteins in ovarian cancers is associated with a poorer prognosis. Between 30% and 50% of advanced-stage ovarian cancers express p53, and expression of the protein is associated with decreased survival.^{9,23} The HER-2/*neu* protein is overexpressed in 10% to 35% of ovarian cancers, and expression is also associated with poor prognosis.^{24,25} Finally, topoisomerase II α is expressed in approximately 20% of ovarian cancers.²⁵ The presence of topoisomerase II α in the tumor is linked to vascular endothelial growth factor expression and is

also associated with increased International Federation of Gynecology and Obstetrics (FIGO) stage, tumor grade, and decreased survival.²⁶ Thus, immunity directed against these particular proteins, if functional, may have a clinical impact.

Humoral immunity to p53 predicted a survival benefit regardless of stage of disease, CA-125 level at the time of diagnosis, or age of the patient. Humoral immunity is associated with oncogenic protein overexpression and accumulation in tumor cells.^{5,6} Specifically, accumulation of wild-type and mutated p53 in tumor cells of ovarian cancer patients is strongly correlated with presence of a p53-specific antibody response.⁵ In addition, an increase in p53-specific antibody response is associated with an increase in tumor volume in p-53 positive colorectal cancers.⁶ Similar results have been found for HER-2/*neu*-positive colorectal cancers with presence of antibodies to HER-2/*neu* correlating to HER-2/*neu* overexpression in primary tumor.²⁷ Humoral immunity to p53 has been found in patients with a variety of gynecologic malignancies.²⁰ However, the relationship between circulating p53 antibodies and prognosis has been unclear. One investigation determined that there was decreased survival in patients with ovarian cancer who had detectable p53 antibodies.²⁸ Such data may suggest that circulating antibodies to p53 are merely a marker of the presence of p53 mutations in the tumor; thus, a poor prognosis. The Danish Malignant Ovarian Cancer (MALOVA) study, which evaluated p53-specific immunity in 193 women with ovarian cancer, found no significant correlation between p53 antibody immunity and outcome.²⁹ Investigations of antibody immunity in ovarian cancer have been hampered by the small numbers of patient samples available, because ovarian cancer is a relatively uncommon disease, as well as by the relative insensitivity of the assays used. For these reasons, most studies focus on limited numbers of patients who have detectable antibodies. For example, only 15% of women with stage III ovarian cancer in the Danish study had evidence of p53-specific antibodies.²⁹ The development of clinical-grade ELISA assays with sensitive limits of detection to evaluate tumor-specific antibody immunity resulted in our ability to detect p53 antibodies in nearly one third of patients.

The statistical power of studies examining tumor-specific immunity in ovarian cancer, a disease with relatively low incidence, remains an issue in determining the association between survival and p53 humoral immunity. We improved the ability of our study to detect differences in survival by increasing assay sensitivity and follow-up time. While the increase we found in overall survival for p53 antibody-positive patients was statistically significant (95% CI, 1.52 to 4.18; $P = .01$) and survival differences for HER-2/*neu* antibody-positive patients and topoisomerase II α antibody-positive patients were not, the 95% CI for HER-2/*neu* was 1.02 to 3.31, and for topoisomerase II α , the 95% CI was 0.206 to 1.65. Given the limited power of this study, we cannot rule out the possibility that true differences do exist. The possibility of a predictive relationship between humoral immunity to these antigens and survival in ovarian cancer patients remains open to further study.

Finally, we found that the differences in survival were specific to immunity mounted against tumor-associated antigens rather than a reflection of general immune competence. A concern of investigations evaluating immunity against cancer and correlating results with disease outcome is that the ability to mount an immune response may be reflective of performance status (ie, the healthier the patient, the higher the likelihood of developing

immunity). Excellent performance status in itself predicts better outcome.^{30,31} To address this question, we evaluated immunity to TT in patients as a control antigen. Approximately 70% of the adult population has detectable antibodies to tetanus, so the evaluation of immunity to tetanus would result in patients who tested both positive and negative for the antibody.³² Antibody immunity against TT was not a predictor of survival. In addition, when tetanus-specific antibodies were present, the level of tetanus-specific antibody immunity detected in these women was no different than that detected in non-cancer bearing individuals (data not shown). These data imply that the survival benefit observed

with tumor antigen-specific immunity was independent from an overall benefit related to general immune competence.

Women with ovarian cancer mount an immune response against their tumors, and evidence is accumulating that indicates that such immune responses may impart a survival benefit. Although current treatment paradigms, such as surgery and adjuvant chemotherapy, have improved survival in patients with advanced-stage ovarian cancer, less than half of patients diagnosed will be cured of their disease. Clinical strategies designed to either augment existent tumor-specific immunity or generate such immunity in women with ovarian cancer may be of therapeutic benefit.

REFERENCES

1. Gnjjatic S, Atanackovic D, Jager E, et al: Survey of naturally occurring CD4+ T cell responses against NY-ESO-1 in cancer patients: Correlation with antibody responses. *Proc Natl Acad Sci U S A* 100:8862-8867, 2003
2. Zhang L, Conejo-Garcia JR, Katsaros D, et al: Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. *N Engl J Med* 348:203-213, 2003
3. Spiotto MT, Fu YX, Schreiber H: Tumor immunity meets autoimmunity: Antigen levels and dendritic cell maturation. *Curr Opin Immunol* 15:725-730, 2003
4. Disis ML, Pupa SM, Gralow JR, et al: High titer HER-2/neu protein specific antibody immunity can be detected in patients with early stage breast cancer. *J Clin Oncol* 15:3363-3367, 1997
5. Vogl FD, Stickeler E, Weyermann M, et al: p53 autoantibodies in patients with primary ovarian cancer are associated with higher age, advanced stage and a higher proportion of p53-positive tumor cells. *Oncology* 57:324-329, 1999
6. Broll R, Duchrow M, Oevermann E, et al: p53 autoantibodies in sera of patients with a colorectal cancer and their association to p53 protein concentration and p53 immunohistochemistry in tumor tissue. *Int J Colorectal Dis* 16:22-27, 2001
7. Nielsen CH, Hegedus L, Leslie RG: Autoantibodies in autoimmune thyroid disease promote immune complex formation with self antigens and increase B cell and CD4+ T cell proliferation in response to self antigens. *Eur J Immunol* 34:263-272, 2004
8. Jager D, Taverna C, Zippelius A, et al: Identification of tumor antigens as potential target antigens for immunotherapy by serological expression cloning. *Cancer Immunol Immunother* 53:144-147, 2004
9. Nielsen JS, Jakobsen E, Holund B, et al: Prognostic significance of p53, Her-2, and EGFR overexpression in borderline and epithelial ovarian cancer. *Int J Gynecol Cancer* 14:1086-1096, 2004
10. Dogan E, Saygili U, Tuna B, et al: p53 and mdm2 as prognostic indicators in patients with

epithelial ovarian cancer: A multivariate analysis. *Gynecol Oncol* 97:46-52, 2005

11. Hogdall EV, Christensen L, Kjaer SK, et al: Distribution of HER-2 overexpression in ovarian carcinoma tissue and its prognostic value in patients with ovarian carcinoma: From the Danish MALOVA Ovarian Cancer Study. *Cancer* 98:66-73, 2003
12. Gotlieb WH, Goldberg I, Weisz B, et al: Topoisomerase II immunostaining as a prognostic marker for survival in ovarian cancer. *Gynecol Oncol* 82:99-104, 2001
13. Goodell V, Disis ML: Human tumor cell lysates as a protein source for the detection of cancer antigen-specific humoral immunity. *J Immunol Methods* 299:129-138, 2005
14. Disis ML, Schiffman K, Guthrie K, et al: Effect of dose on immune response in patients vaccinated with an HER-2/neu intracellular domain protein-based vaccine. *J Clin Oncol* 22:1916-1925, 2004
15. Nakatsura T, Senju S, Ito M, et al: Cellular and humoral immune responses to a human pancreatic cancer antigen, coactosin-like protein, originally defined by the SEREX method. *Eur J Immunol* 32:826-836, 2002
16. Di Modugno F, Bronzi G, Scanlan MJ, et al: Human Mena protein, a serex-defined antigen overexpressed in breast cancer eliciting both humoral and CD8+ T-cell immune response. *Int J Cancer* 109:909-918, 2004
17. Balkwill F: Cancer and the chemokine network. *Nat Rev Cancer* 4:540-550, 2004
18. Zou W: Immunosuppressive networks in the tumour environment and their therapeutic relevance. *Nat Rev Cancer* 5:263-274, 2005
19. Loercher AE, Nash MA, Kavanagh JJ, et al: Identification of an IL-10-producing HLA-DR-negative monocyte subset in the malignant ascites of patients with ovarian carcinoma that inhibits cytokine protein expression and proliferation of autologous T cells. *J Immunol* 163:6251-6260, 1999
20. Soussi T: p53 Antibodies in the sera of patients with various types of cancer: A review. *Cancer Res* 60:1777-1788, 2000
21. Fisk B, Blevins TL, Wharton JT, et al: Identification of an immunodominant peptide of HER-2/neu protooncogene recognized by ovarian tumor-specific cytotoxic T lymphocyte lines. *J Exp Med* 181:2109-2117, 1995

22. Stone B, Schummer M, Paley PJ, et al: Serologic analysis of ovarian tumor antigens reveals a bias toward antigens encoded on 17q. *Int J Cancer* 104:73-84, 2003

23. Bali A, O'Brien PM, Edwards LS, et al: Cyclin D1, p53, and p21Waf1/Cip1 expression is predictive of poor clinical outcome in serous epithelial ovarian cancer. *Clin Cancer Res* 10:5168-5177, 2004
24. Camilleri-Broet S, Hardy-Bessard AC, Le Tourneau A, et al: HER-2 overexpression is an independent marker of poor prognosis of advanced primary ovarian carcinoma: A multicenter study of the GINECO group. *Ann Oncol* 15:104-112, 2004
25. Mano MS, Awada A, Di Leo A, et al: Rates of topoisomerase II-alpha and HER-2 gene amplification and expression in epithelial ovarian carcinoma. *Gynecol Oncol* 92:887-895, 2004
26. Brustmann H: Vascular endothelial growth factor expression in serous ovarian carcinoma: Relationship with topoisomerase II alpha and prognosis. *Gynecol Oncol* 95:16-22, 2004
27. Ward RL, Hawkins NJ, Coomber D, et al: Antibody immunity to the HER-2/neu oncogenic protein in patients with colorectal cancer. *Hum Immunol* 60:510-515, 1999
28. Abendstein B, Marth C, Muller-Holzner E, et al: Clinical significance of serum and ascitic p53 autoantibodies in epithelial ovarian carcinoma. *Cancer* 88:1432-1437, 2000
29. Hogdall EV HC, Blaakaer J, et al: p53 autoantibodies in sera from Danish ovarian cancer patients and their correlation with clinical data and prognosis. *APMIS*:7-8, 2002
30. Cella D: The Functional Assessment of Cancer Therapy-Lung and Lung Cancer Subscale assess quality of life and meaningful symptom improvement in lung cancer. *Semin Oncol* 31:11-15, 2004
31. Chau I, Norman AR, Cunningham D, et al: Multivariate prognostic factor analysis in locally advanced and metastatic esophago-gastric cancer-pooled analysis from three multicenter, randomized, controlled trials using individual patient data. *J Clin Oncol* 22:2395-2403, 2004
32. Gergen PJ, McQuillan GM, Kiely M, et al: A population-based serologic survey of immunity to tetanus in the United States. *N Engl J Med* 332:761-766, 1995

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Glossary

Cytokines: Cell communication molecules that are secreted in response to external stimuli.

Humoral: Pertaining to elements in the blood or other body fluids.

Immunity: Resistance to invasion by a specific pathogen.

Immunogenic: Capable of inducing an immune response.

Immunoglobulin: A class of proteins produced in lymph tissue.

Immunosuppressive: A substance that lowers the body's normal immune response.