Steroid Hormone Regulation and Prognostic Value of the Human Kallikrein Gene 14 in Ovarian Cancer

George M. Yousef, MD, PhD,[1,2] Stefano Fracchioli, MD,[3] Andreas Scorilas, PhD,[4] Carla A. Borgoño,[1,2] Lisa Iskander,[2] Manuela Puopolo, MD,[3] Marco Massobrio, MD,[3] Eleftherios P. Diamandis, MD, PhD, FRCP,[1,2] and Dionyssios Katsaros, MD[3]

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Abstract

To study KLK14 *gene expression in endocrine-related cancers, we studied its hormonal regulation in breast and ovarian cancer cell lines. Our kinetic and blocking experiments suggest that this up-regulation is mediated through the androgen receptor. We then studied the expression of* KLK14 *by quantitative reverse transcriptase–polymerase chain reaction in 155 consecutive ovarian tumors and correlated these findings with clinicopathologic parameters, response to chemotherapy, and survival. A stepwise reduction was observed in the levels of* KLK14 *messenger RNA in normal, benign, and cancerous tissues (*P < *.001). Expression levels were significantly higher in patients with early stage disease and optimal debulking and in patients who responded to chemotherapy. Kaplan-Meier survival curves demonstrated longer progression-free and overall survival in patients with* KLK14*-positive tumors than in patients with* KLK14*-negative tumors (*P < *.001). When all other prognostic variables were controlled in the multivariate analysis,* KLK14 *retained its prognostic significance (progression-free and overall survival, respectively, hazard ratios, 0.43 and 0.53;* P = *.027 and .014). A weak negative correlation was found between* KLK14 *expression and serum CA-125.* KLK14 *is a new, independent, and favorable prognostic marker for ovarian cancer.*

Epithelial ovarian carcinoma is the most common and most lethal of all gynecologic malignant neoplasms. Only 30% of ovarian tumors are diagnosed at an early stage (I or II), when survival rates reach 90%. The rest are diagnosed at an advanced stage, with survival rates of less than 20%.[1] Currently, the only well-accepted serologic marker is CA-125, a large glycoprotein of unknown function.[2] CA-125 has serious limitations as a diagnostic, prognostic, and screening tool.[3] Consequently, there is a need to develop new biomarkers that can assist in identifying the prognosis and progression of this malignant neoplasm, reaching treatment decisions, monitoring response after treatment, and identifying relapse during routine follow-up. Several putative markers have been sought to compensate for the limitations of CA-125, including inhibin, prostasin, OVX1, LASA, CA-15.3 and CA-72-4.[4-9] Although their relevance in the management of ovarian carcinoma is yet to be determined, these novel markers may by used in combination with CA-125, thereby enhancing the overall diagnostic and prognostic capabilities.[10]

Kallikreins are a subgroup of secreted serine proteases, encoded by highly conserved and tightly clustered multigene families in humans, rats, and mice. The human kallikrein gene family resides on chromosome 19q13.4 and includes 15 members, whose genes are designated as *KLK1* to *KLK15* and the corresponding proteins as hK1 to hK15.[11-13] Kallikreins are expressed in a wide variety of tissues and are found in many biologic fluids (eg, cerebrospinal fluid, serum, seminal plasma, milk) in which they are believed to process specific substrates. Kallikreins may participate in cascade reactions similar to those involved in digestion, fibrinolysis, coagulation, wound healing, and

apoptosis.[11] Many kallikreins have been found to be expressed differentially in endocrine-related malignant neoplasms,[14] including prostate,[15-17] ovarian,[18-23] breast,[24-26] and testicular cancer.[27] In addition, many kallikrein genes examined thus far are under steroid hormone regulation, implying a role for kallikreins in endocrine-related tissues.[11] Furthermore, hK6, hK10, and hK11 recently have been identified as novel serologic ovarian cancer biomarkers.[23,28,29]

Yousef et al[24] cloned a novel kallikrein gene, *KLK14* (formerly known as *KLK-L6*). This gene has a restricted tissue expression pattern and is found in the central nervous system, particularly cerebrum, cerebellum, and spinal cord, as well as in endocrine-related tissues such as the uterus, ovary, thyroid, and testis. Preliminary studies have shown that *KLK14* is down-regulated at the messenger RNA (mRNA) level in prostatic, testicular, ovarian, and breast cancer tissues (compared with normal tissues) and in 2 breast cancer cell lines.[24] In this respect, *KLK14* resembles *KLK3* (prostate-specific antigen) and *KLK10* in breast cancer and *KLK9* in ovarian cancer.[22,30,31] In situ hybridization studies demonstrated that *KLK14* is expressed by the secretory epithelial cells of benign prostate gland, prostatic intraepithelial neoplasia, and malignant prostate cells.[32]

We studied the hormonal regulation of this gene in breast and ovarian cancer cell lines and its value as a prognostic marker in ovarian carcinoma.

Materials and Methods

Breast Cancer Cell Lines and Hormonal Stimulation Experiments

The breast cancer cell lines BT-474, T-47D, ZR-75, T-47D, and BT-20 and the ovarian cancer cell line HTB-75 (Caov-3) were purchased from the American Type Culture Collection, Rockville, MD. The BG-1 ovarian cancer cell line was provided by Henri Rochefort, MD, Montpellier, France. Cells were cultured in RPMI media (Gibco BRL, Gaithersburg, MD) supplemented with glutamine (200-mmol/L concentration) and fetal bovine serum (10%), in plastic flasks, to near confluence. The cells then were divided into aliquots in 24-well tissue culture plates and cultured to 50% confluence. Twenty-four hours before the experiments, the culture medium was replaced with medium containing 10% charcoal-stripped fetal bovine serum. For stimulation experiments, various steroid hormones dissolved in 100% ethanol were added into the culture media at a final concentration of 10^{-8} mol/L. Cells stimulated with 100% ethanol were included as controls. The cells were grown for 24 hours, then harvested for mRNA extraction.

Blocking and Kinetic Experiments

Blocking experiments were performed as follows: (1) addition to cultured cells of androgen receptor (AR) blockers (ie, RU56,187 and nilutamide) individually at 3 concentrations (10^{-6}, 10^{-7}, 10^{-8} mol/L); (2) stimulation by dihydrotestosterone (DHT) alone at a concentration of 10^{-8} to 10^{-10} mol/L; (3) addition of AR blockers at the 3 aforementioned concentrations for 1 hour, followed by DHT stimulation at a concentration 100-fold lower (10^{-8} to 10^{-10} mol/L) than that of the blockers. Ethanol-only–stimulated cells were included as controls to assess baseline *KLK14* expression. Cells were harvested for analysis after 24 hours.

For kinetic experiments, the BT-474 cell line was stimulated by DHT at a final concentration of 10^{-8} mol/L and then harvested at 0 (just before stimulation), 2, 6, 12, and 24 hours. Control cells stimulated with ethanol were included for all time points. All experiments were repeated twice.

Study Population

Included in this study were tumor specimens from 155 consecutive patients undergoing surgical treatment for epithelial ovarian carcinoma at the Department of Gynecology, University of Turin, Turin, Italy. Diagnosis was confirmed by histopathologic examination. Patients received no treatment before surgery.

Patient ages ranged from 19 to 89 years (median, 58 years). Residual tumor size ranged from 0 to 9 cm (median, 1.2 cm). With respect to histologic type, 70 tumors were serous papillary, 28 were endometrioid, 24 were undifferentiated, 15 were mucinous, and 16 were clear cell. For 2 tumors, the histologic type was unknown. We also included 10 normal ovarian tissue samples and 10 tissue samples from ovaries with benign disease, from women whose median ages were 52 and 54 years, respectively. Classification of histologic types was according to World Health Organization criteria.[33] All patients were staged according to the International Federation of Gynecology and Obstetrics staging system.[34] Grading information was available for 148 patients; 53 (35.8%) had grade 1 or 2, and 95 (64.2%) had grade 3 ovarian carcinoma. Grading was established for each ovarian tumor according to the criteria of Day et al.[35]

All patients were treated postoperatively with a platinum-based chemotherapy regimen. The first-line chemotherapy regimens included cisplatin in 87 patients (56.1%), carboplatin in 46 (29.7%), cyclophosphamide in 64 (41.3%), doxorubicin in 11 (7.1%), epirubicin in 18 (11.6%), paclitaxel in 25 (16.1%), and methotrexate in 2 (1.3%). Patients with grade 1 and stage I disease received no further treatment. Response to chemotherapy was defines as follows: *complete response*, resolution of all evidence of disease for at least 1 month; *partial response*, a decrease (lasting at least 1 month) of at least 50% in the diameters of

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all measurable lesions without the development of new lesions; *stable disease*, a decrease of less than 25% in the product of the diameters of all measurable lesions; and *progressive disease*, an increase of at least 25%. Studies were performed in accordance with the Helsinki Declaration and were approved by the review board of the Institute of Obstetrics and Gynecology, Turin, Italy. Tumor specimens were snap-frozen in liquid nitrogen immediately after surgery. Histologic examination, performed during intraoperative frozen section analysis, permitted representative portions of each tumor containing more than 80% tumor cells to be selected for storage until analysis.

Total RNA Extraction and Complementary DNA Synthesis

Samples were shipped and stored at –80°C. They then were minced with a scalpel on dry ice and transferred immediately to 2-mL polypropylene tubes and homogenized. Total RNA was extracted using Trizol reagent (Gibco BRL) according to the manufacturer's instructions. The concentration and purity of RNA were determined spectrophotometrically. Two micrograms of total RNA was reverse-transcribed into first-strand complementary DNA (cDNA) using the Superscript preamplification system (Gibco BRL). The final volume was 20 µL.

Quantitative Real-Time Polymerase Chain Reaction (PCR) and Continuous Monitoring of PCR Products

Based on the published genomic sequence of *KLK14* (Genbank accession No. AF161221), 2 gene-specific primers were designed (6F5: 5' AGT GGG TCA TCA CTG CTG CT 3' and 6R5: 5' TCG TTT CCT CAA TCC AGC TT 3'). These primers spanned more than 2 exons to avoid contamination by genomic DNA. Real-time monitoring of the PCR reaction was performed on the LightCycler system (Roche Molecular Systems, Indianapolis, IN), and SYBR Green I dye (Roche Molecular Systems), which binds preferentially to double-stranded DNA, was used. Fluorescence signals are proportional to the concentration of the product and are measured at the end of each cycle rather than after a fixed number of cycles. The higher the starting quantity of the template, the earlier the *threshold cycle*, defined as the fractional cycle number at which fluorescence passes a fixed threshold above baseline.[36] For each sample, the amount of *KLK14* and of an endogenous control (beta-actin, a housekeeping gene) were determined using a calibration curve (see "Standard Curve Construction"). The amount of *KLK14* then was divided by the amount of the endogenous reference material to obtain a normalized *KLK14* value.

Standard Curve Construction

The full-length mRNA sequence of the *KLK14* gene was amplified by PCR using gene-specific primers, and the PCR product was cloned into a TOPO TA cloning vector (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. A plasmid containing beta-actin cDNA was prepared similarly. Plasmids were purified using a miniprep kit (Qiagen, Valencia, CA). Different standard curves for actin and *KLK14* were constructed using serial dilutions of the plasmid as described elsewhere.[36] These standards were included in each run.

PCR Amplification

The PCR reaction was carried out on the LightCycler system. A calibration curve was created from serial dilutions of a total RNA preparation from ovarian tissue (10-fold at a time), and an arbitrary copy number was assigned to each sample, according to the dilution factor. Each calibration and patient sample were analyzed in duplicate, and sample values were calculated according to the calibration curve that was included in each cycle. For each run, a master mixture containing 1 µL of cDNA, 2 µL of LC DNA Master SYBR Green 1 mix, 50 ng of primers, and 2.4 µL of a 25-mmol/L concentration of magnesium chloride was prepared on ice. After loading the reaction mixture into glass capillary tubes, cycling conditions were carried out as shown in **Table 1**. To verify the melting curve results, representative PCR products were sequenced.

Statistical Analysis

First, an optimal cutoff value was defined by chi-square analysis, based on the ability of *KLK14* values to predict the progression-free and overall survival of the study population. This cutoff (1.0 arbitrary units; 50th percentile) identifies 50% of patients as being positive for *KLK14*.

Associations between clinicopathologic parameters such as stage, grade, histologic type, and residual tumor, and *KLK14* expression were analyzed by using the chi-square test or the Fisher exact test, where appropriate. For survival analysis, 2 different endpoints, cancer relapse (local recurrence or distant metastasis) and death, were used to calculate progression-free and overall survival, respectively. *Progression-free survival* was defined as the interval between the date of surgery and the date of identification of recurrent or metastatic disease. *Overall survival* was defined as the interval between the date of surgery and the date of death.

The Cox univariate and multivariate proportional hazards regression models[37] were used to evaluate the hazard ratio (relative risk of relapse or death in the *KLK14*-positive group). In multivariate analysis, the models were adjusted for *KLK14* expression, clinical stage, histologic grade, residual tumor, and age.

Kaplan-Meier survival curves[38] were constructed for *KLK14*-positive and *KLK14*-negative patients. For further analysis, patients were divided into 2 groups by tumor grade

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Table 1
Experimental Protocol Used for Quantitative PCR Amplification of the *KLK14* Gene

Protocol Step/Segment No.	Temperature Target (°C)	Hold Time (s)	Slope (°C/s)	Application Mode
Denaturation (1 cycle)				
1	95	600	20	None
PCR (35 cycles)				
1	95	0	20	None
2	62	5	20	None
3	72	40	20	None
4	85	5	20	Single
Melting (1 cycle)				
1	95	0	20	None
2	72	30	20	None
3	97	0	0.2	Step
Cooling (1 cycle)				
1	40	30	1	None

PCR, polymerase chain reaction.

(grade 1-2 vs grade 3), tumor stage (stage I-II vs stage III-IV), or by the success of debulking (optimal vs suboptimal). In each category, survival rates (progression-free and overall survival) were compared between *KLK14*-positive and *KLK14*-negative groups. The differences between survival curves were analyzed by using the log-rank test.[39]

Results

Hormonal Regulation of the *KLK14* Gene

Sequence analysis of the *KLK14* gene promoter using different promoter prediction algorithms indicated the presence of a putative androgen response element (GGAGGCAAGCAGCCTC) at position –386 (base numbering according to our Genbank accession No. AF161221). This element is comparable to the androgen response element II of the prostate-specific antigen promoter, which is found at approximately the same position. Thus, we postulated that *KLK14* is regulated by androgens, as is the case with some other kallikreins.

To test this hypothesis, we examined *KLK14* gene expression in 4 breast cancer cell lines (BT-474, ZR-75, T-47D, and BT-20) and 2 ovarian cancer cell lines (BG-1 and HTB-75 [Caov-3]) with variable AR content. Quantitative PCR results indicated that *KLK14* was mainly up-regulated by androgens (DHT) in the AR-positive breast cancer cell lines (BT-474, T-47D, and ZR-75) and to a lesser extent by progestins **Figure 1**. The gene also was up-regulated by

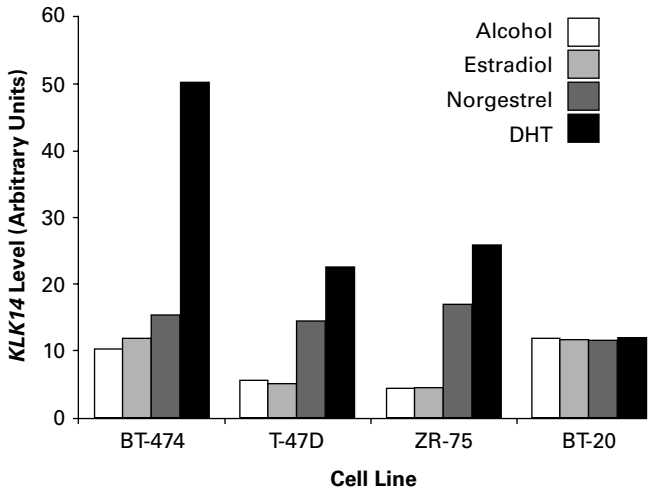

Figure 1 *KLK14* messenger RNA concentration in 4 breast cancer cell lines 24 hours after stimulation with steroids at a concentration of 10^{-8} mol/L. DHT, dihydrotestosterone.

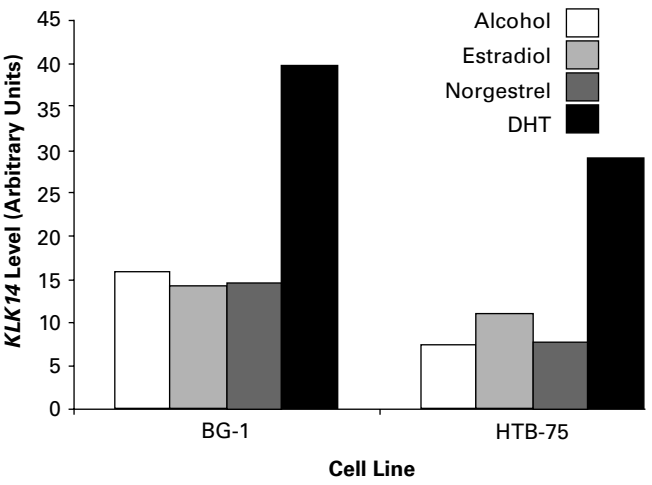

Figure 2 *KLK14* messenger RNA concentration in 2 ovarian cancer cell lines 24 hours after stimulation with steroids at a concentration of 10^{-8} mol/L. DHT, dihydrotestosterone.

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DHT in both ovarian cancer cell lines **Figure 2**. No significant up-regulation of *KLK14* was found in the AR-negative cell line BT-20 (Figure 1).

Time-course experiments indicated that *KLK14* gene expression started to increase as early as 2 hours after hormonal stimulation, and mRNA levels increased steadily during the next 24 hours **Figure 3**. Results of the blocking experiments are represented in **Figure 4**. Both antiandrogens used, RU56,187 and nilutamide, were added in 100-fold excess before DHT stimulation. Nilutamide was able to block the stimulatory effect of DHT by about 50%. RU56,187 had little effect on androgenic stimulation (data not shown).

KLK14 Expression in Normal, Benign, and Ovarian Cancer Tissue Samples

A comparison of *KLK14* expression in normal ovarian tissues, benign ovarian tumors, and cancerous ovarian tissues is shown in **Table 2** and **Figure 5**. A stepwise reduction in *KLK14* mRNA levels was observed between normal tissues (median = 74 arbitrary units), benign ovarian tumors (median = 6.5 arbitrary units), and cancerous tissues (median = 1.0 arbitrary units). These differences were statistically significant ($P < .001$).

KLK14 Expression in Relation to Other Variables

As shown in **Table 3**, *KLK14* expression levels were significantly higher in patients with early stage (I or II) disease ($P = .020$), in patients with optimal debulking ($P = .020$), and in patients with a better response to chemotherapy ($P = .008$). No significant associations were found between *KLK14* expression and grade, histologic type, residual tumor, and menopausal status.

Survival Analysis

Of the 155 patients included in the study, follow-up information was available for 147, among whom 79 (53.7%) experienced relapse and 50 (34.0%) died. Kaplan-Meier survival curves demonstrated longer progression-free and overall survival ($P < .001$ for both) for patients with *KLK14*-positive tumors than for patients with *KLK14*-negative

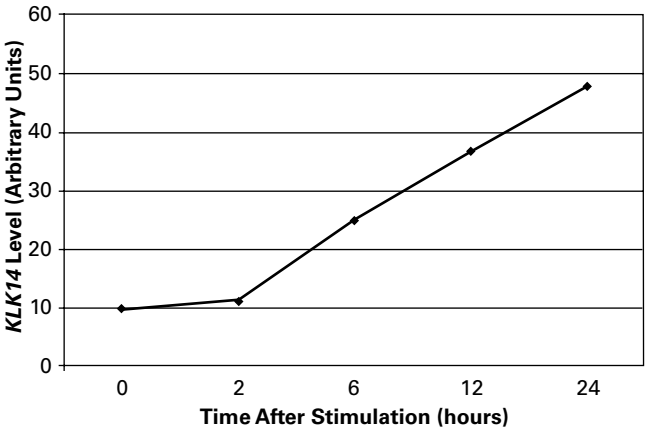

Figure 3 *KLK14* messenger RNA concentration in the BT-474 breast cancer cell line before (0) and at 2, 6, 12, and 24 hours after stimulation with dihydrotestosterone at a concentration of 10^{-8} mol/L.

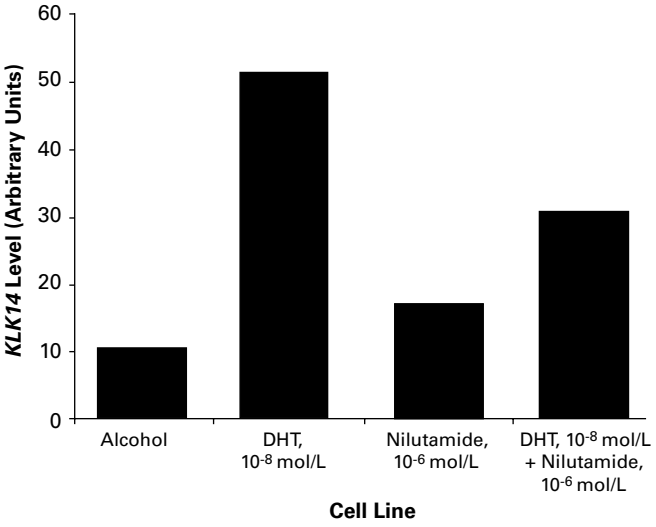

Figure 4 *KLK14* messenger RNA concentration in the BT-474 cell line 24 hours after dihydrotestosterone (DHT) stimulation with and without blocking by nilutamide. Nilutamide was added at a concentration of 10^{-6} mol/L, and DHT was added 1 hour later at a concentration of 10^{-8} mol/L . Alcohol was added as a control.

Table 2
Descriptive Statistics for *KLK14* Expression in Cancerous, Benign, and Normal Ovarian Tissues*

		Percentiles				
Ovarian Tissue Type	**Range**	**10**	**25**	**50 (Median)**	**75**	**90**
Cancerous (n = 155)	0.01-97,643	0.011	0.06	1.00	22	128
Benign tumor (n = 10)	0.17-5,000	0.32	1.75	6.5	98	4,510
Normal (n = 10)	3.2-11,000	3.9	40	74	298	9,913

* *KLK14* expression is given in arbitrary units.

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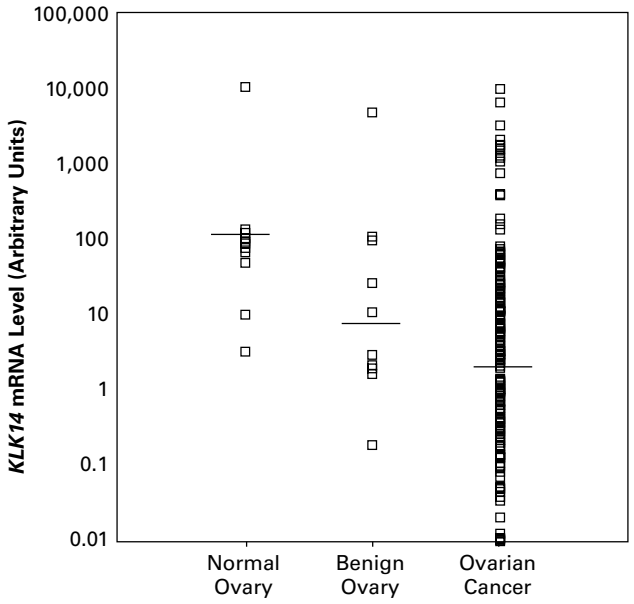

Figure 5 Distribution of *KLK14* messenger RNA concentration in normal ovarian tissues (n = 10), benign ovarian tumors (n = 10), and ovarian cancer tissues (n = 155). The horizontal lines represent median values. $P < .001$; Kruskal-Wallis test.

tumors **Figure 6**. The strength of the associations between each individual prognostic factor and progression-free or overall survival is shown in the univariate analysis in **Table 4**. Stage of disease, histologic grade, and residual tumor size showed strong associations with cancer relapse and death ($P < 0.001$). *KLK14* expression also was a significant predictor of prolonged progression-free and overall survival (hazard ratios, 0.38 and 0.33, respectively; $P < .001$ for both).

In Cox multivariate analysis, only the residual tumor size and grade, in addition to *KLK14* expression, retained their prognostic significance (Table 4).

Survival analyses also were performed for the subgroups of patients stratified by histologic type **Table 5**. No statistically significant differences were found in any subgroup except for a highly significant difference in the progression-free survival for patients in the endometrioid subgroup ($P = .007$).

As shown in **Figure 7**, a weak negative correlation was found between preoperative serum CA-125 and *KLK14* mRNA levels ($r_s = –0.28$; $P = .024$).

Table 3
Relationship Between *KLK14* Status and Other Variables in 155 Patients With Ovarian Cancer*

Variable (No. of Cases)	*KLK14* Status		*P*
	Negative	Positive	
Stage			.020†
I/II (n = 45)	16 (36)	29 (64)	
III/IV (n = 103)	59 (57.3)	44 (42.7)	
Status unknown (n = 7)	—	—	
Grade			.23†
1/2 (n = 53)	23 (43)	30 (57)	
3 (n = 95)	52 (55)	43 (45)	
Status unknown (n = 7)	—	—	
Histologic type			.30‡
Serous (n = 70)	39 (56)	31 (44)	
Endometrioid (n = 28)	9 (32)	19 (68)	
Mucinous (n = 15)	10 (67)	5 (33)	
Clear cell (n = 16)	10 (63)	6 (38)	
Undifferentiated (n = 24)	11 (46)	13 (54)	
Status unknown (n = 2)	—	—	
Residual tumor (cm)			.11‡
0 (n = 67)	28 (42)	39 (58)	
1-2 (n = 27)	17 (63)	10 (37)	
>2 (n = 53)	30 (57)	23 (43)	
Status unknown (n = 8)	—	—	
Debulking success			.020†
Optimal (0-1 cm) (n = 79)	33 (42)	46 (58)	
Suboptimal (>1 cm) (n = 68)	42 (62)	26 (38)	
Status unknown (n = 8)	—	—	
Menopause			.29†
Premenopause or perimenopause (n = 48)	21 (44)	27 (56)	
Postmenopause (n = 105)	57 (54.3)	48 (45.7)	
Status unknown (n = 2)	—	—	
Response to chemotherapy			.008†
Complete or partial response (n = 125)	58 (46.4)	67 (53.6)	
No change or progressive disease (n = 17)	14 (82)	3 (18)	
Not evaluated (n = 13)	—	—	

* Data are given as number (percentage).
† Fisher exact test.
‡ Chi-square test.

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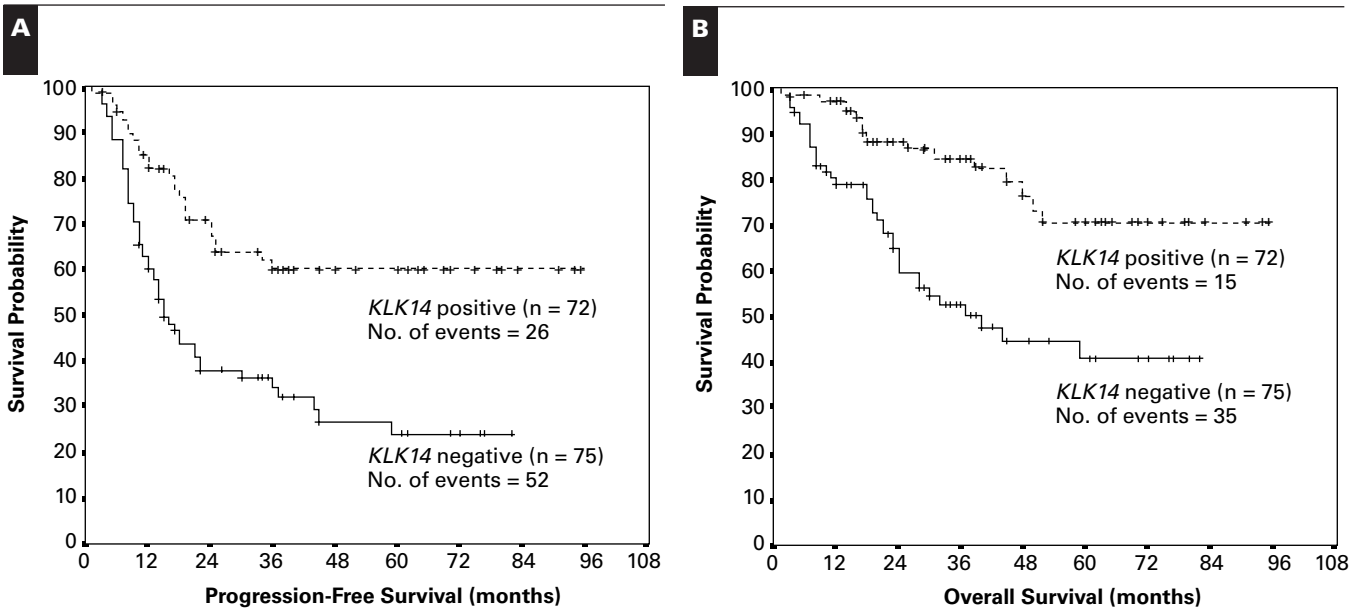

Figure 6 Kaplan-Meier survival curves for the progression-free survival (**A**) and overall survival (**B**) in patients with *KLK14*-positive and *KLK14*-negative ovarian tumors. **A** and **B**, $P < .001$.

Discussion

As is the case with other kallikrein genes, our results show that *KLK14* expression is under steroid hormonal regulation. *KLK14* is mainly up-regulated by androgens and progestins in breast and ovarian cancer cell lines. We also provide some indirect evidence that this regulation might be mediated through the AR. Ovarian cancer is an endocrine-related malignant neoplasm, and compelling evidence supports the role of steroid hormones in the development and progression of this disease.[40,41] In addition, the AR gene represents a plausible candidate genetic modifier of ovarian cancer risk.[42] Identification of downstream AR-regulated genes is an important initial step toward understanding the

Table 4
Univariate and Multivariate Analysis of *KLK14* With Regard to Progression-Free and Overall Survival*

	Survival					
	Progression-Free			Overall		
Variable	Hazard Ratio	95% CI	*P*	Hazard Ratio	95% CI	*P*
Univariate analysis (n = 147)						
Negative	1.00	—	—	1.00	—	—
Positive	0.38	0.24-0.62	< .001	0.33	0.18-0.61	< .001
As a continuous variable	0.99	0.99-1.00	.48	0.99	0.99-1.00	.78
Stage of disease (ordinal)	2.48	1.88-3.25	< .001	2.51	1.76-3.57	< .001
Grading (ordinal)	2.068	1.57-2.72	< .001	2.13	1.46-3.09	< .001
Residual tumor (ordinal)	1.27	1.21-1.33	< .001	1.30	1.21-1.39	< .001
Histologic type†	1.48	1.00-2.18	.046	1.29	0.79-2.11	.30
Age	1.01	0.99-1.03	.12	1.01	0.99-1.03	.13
Multivariate analysis (n = 144)						
Negative	1.00	—	—	1.00	—	—
Positive	0.53	0.31-0.93	.027	0.42	0.21-0.84	.014
As a continuous variable	0.99	0.98-1.00	.15	0.99	0.98-1.01	.20
Stage of disease (ordinal)	1.38	0.89-2.14	.14	1.57	0.91-2.73	.11
Grading (ordinal)	1.61	1.02-2.56	.041	1.35	0.75-2.41	.30
Residual tumor (ordinal)	1.21	1.09-1.33	< .001	1.31	1.16-1.48	< .001
Histologic type†	1.04	0.86-1.25	.65	1.26	1.01-1.58	.035
Age	1.02	0.99-1.04	.15	1.02	0.99-1.05	.13

* The hazard ratios were estimated from a Cox proportional hazards regression model. The confidence interval (CI) is of the estimated hazard ratio.
† Serous vs others.

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Table 5
Survival Analysis of *KLK14* Messenger RNA for Subgroups of Histologic Types*

	Survival					
	Progression-Free			Overall		
Variable	Hazard Ratio	95% CI	*P*	Hazard Ratio	95% CI	*P*
Serous	0.57	0.29-1.15	.12	0.51	0.19-1.35	.18
Endometrioid	0.14	0.033-0.58	.007	0.14	0.014-1.35	.09
Mucinous	0.59	0.062-5.81	.65	0.44	0.09-2.18	.32
Clear cell	0.47	0.09-2.41	.37	0.38	0.044-3.26	.37
Undifferentiated	0.41	0.15-1.13	.09	0.35	0.11-1.09	.07

* The hazard ratios were estimated from a Cox proportional hazards regression model. The confidence interval (CI) is of the estimated hazard ratio.

mechanism by which androgens are implicated in ovarian cancer. These findings may have therapeutic applications.

Our results show that *KLK14* is an independent marker of favorable prognosis in ovarian cancer (Table 4). *KLK14* is not the only kallikrein that has been found to be regulated differentially in ovarian cancer. *KLK9* is also a marker of favorable prognosis.[22] In addition, data from other groups and from our laboratory indicate that other kallikrein genes (*KLK4-KLK10*) all are expressed differentially in ovarian cancer.[18,20,21,23,28,43]

It is now widely accepted that no single biomarker will provide all the necessary information for diagnosis, prognosis, and development of treatment strategies for patients with ovarian and other cancers. Instead, research is focussing on devising panels of ovarian cancer biomarkers.

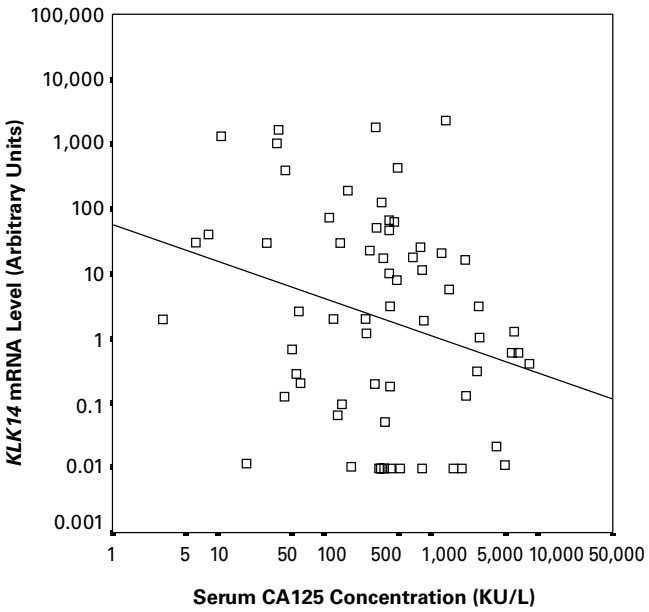

Figure 7 Correlation between serum CA-125 and tumor levels of *KLK14* messenger RNA. $r_s = -0.28$ (Spearman correlation coefficient); $P = .024$.

Artificial network and other combinatorial approaches seem to be promising in this regard.[44-46] It will be interesting to examine the expression of all these kallikreins in ovarian cancer and to determine whether their combination constitutes a more powerful prognostic or predictive panel. In addition, *KLK14* might find applicability as a predictive marker for therapy, similar to steroid receptors and hormonal therapy or HER-2 and trastuzumab (Herceptin) therapy in breast cancer.[47]

Our data indicate that *KLK14* expression levels are correlated negatively with serum CA-125 concentration (Figure 7). These results are consistent with previous reports showing that higher serum CA-125 levels are associated with poor prognosis in ovarian cancer.[48] On the other hand, while high CA-125 expression levels are associated with the serous histologic type,[48] relationships between *KLK14* levels and histologic type were not found (Table 3).

We report for the first time that higher *KLK14* expression has favorable prognostic value in ovarian cancer. These data add to the growing literature suggesting that many other members of the kallikrein gene family have prognostic value in ovarian cancer. It is conceivable that all these enzymes may participate in a common pathway that is activated during ovarian cancer initiation and progression.

From the Departments of [1]Pathology and Laboratory Medicine, Mount Sinai Hospital, [2]Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada; and [3]Obstetrics and Gynecology, Gynecologic Oncology Unit, University of Turin, Turin, Italy; and the [4]National Center of Scientific Research "Demokritos", Athens, Greece.

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Address reprint requests to Dr Diamandis: Dept of Pathology and Laboratory Medicine, Mount Sinai Hospital, 600 University Ave, Toronto, Ontario, Canada M5G 1X5.

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