


ORIGINAL RESEARCH

Insulin-like growth factor 2: a poor prognostic biomarker linked to racial disparity in women with uterine carcinosarcoma

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Introduction

Uterine carcinosarcoma (UCS) is a rare but highly aggressive uterine malignancy that accounts for a disproportionate number of endometrial cancer deaths [1]. These biphasic

Abstract

The objective of this study was to investigate the relationship of insulin-like growth factor 2 (IGF2) expression and survival in women with uterine carcinosarcoma (UCS). Insulin-like growth factor 2 protein expression was determined by immunohistochemical staining of tumor tissues from 103 patients with UCS. The H-score (product of staining intensity and percentage positive cells) was quantified for the epithelial cytoplasmic (EC), epithelial nuclear (EN), and malignant stromal compartments. Multivariable Cox proportional hazard regression models were used to examine the relationship of IGF2 levels with progression-free survival (PFS) and overall survival (OS). Adjusting for stage, race, and adjuvant therapy, PFS and OS were reduced in patients with high IGF2 (H-score \geq median) in the EC and EN compartments. Black race was independently associated with reduced PFS and OS in patients with early-stage disease, and IGF2 levels in the EC were higher in black than in white patients ($P = 0.02$, Wilcoxon test). In a race-stratified multivariable analysis, high IGF2 in the epithelial compartments more than doubled the risk of death in black women; HR = 2.43 (95% CI: 1.18–5.01, $P = 0.02$) for high IGF2 in the EC; and HR = 2.34 (95% CI: 1.25–4.39, $P = 0.008$) for high IGF2 in the EN. In conclusion, high tumor IGF2 expression is an independent risk factor for reduced PFS and OS in UCS. Black women have elevated tumor IGF2 compared with white women, and decreased survival associated with high IGF2. These findings identify IGF2 as a candidate biomarker for survival linked to racial disparity in women with UCS.

tumors are composed of carcinomatous and sarcomatous elements originating from a common progenitor cell [2]. Mutations in chromatin remodeling genes and histones are implicated in the tumorigenesis of carcinosarcoma, characterized by sarcomatous transformation of carcinoma

cells via epithelial mesenchymal transition (EMT) [2–5]. Black women have more than a twofold higher incidence of UCS compared to white women, as well as higher mortality [6–8]. Five-year survival rates are approximately 30% for all women with UCS, and 50–60% for patients with disease confined to the uterus [9].

The insulin-like growth factor 2 (*IGF2*) gene is located on chromosome 11 and is one of the few hundred imprinted genes in the genome. Imprinting is maintained by epigenetic mechanisms, mainly DNA methylation [10]. *IGF2* promoters are developmentally regulated and selectively repressed in adult tissue types [11]. *IGF2* upregulation has been observed in many childhood and adult malignancies. *IGF2* overexpression occurs in the majority of sarcomas, often associated with loss of imprinting at the *IGF2* locus, and approximately 50% of uterine leiomyosarcomas have high *IGF2* expression [12]. Loss of *IGF2* imprinting has also been observed in UCS tumor tissue [13], and nonislet cell hypoglycemia due to tumor production of incompletely processed forms of pro-*IGF2* (“big” *IGF2*) was previously reported in patients with UCS [14], supporting a potential, unrecognized role of *IGF2* in UCS.

The insulin-like growth factor (IGF) family includes ligands (*IGF1* and *IGF2*), tyrosine kinase receptors (*IGF1R* and *IGF2R*), and IGF binding proteins. The interaction between the ligands and the receptors is a complex, dynamic interplay [10, 15]. IGF ligands as well as insulin bind to IGF receptors, insulin receptors, and hybrid receptors, leading to tyrosine autophosphorylation and activation. Receptor activation leads to phosphorylation of insulin receptor substrate and Src homology 2 domain-containing transforming protein 1 (SHC). Phosphorylation of these receptor substrates results in activation of the phosphatidylinositol 3-kinase/AKT (PI3K/AKT) and mitogen-activated protein kinase (MAPK) signaling cascades [16]. These signaling cascades inhibit apoptosis and promote cellular proliferation. Reactivation of fetal *IGF2* promoters and *IGF2* overexpression is associated with worse prognosis in epithelial ovarian cancer and other cancers [17–19]. Dysregulation of the IGF signaling pathway is linked to EMT, a major driver of metastasis and drug resistance and a defining feature of UCS [15].

The objective of our study was to evaluate tumor *IGF2* expression in a cohort of patients with UCS and determine the relationship of *IGF2* with clinicopathologic risk factors, and with disease progression and survival.

Methods

This study was reviewed and approved by the Institutional Review Boards of the Albert Einstein College of Medicine and the University of Alabama at Birmingham. Formalin-fixed, paraffin-embedded (FFPE) tumor specimens were

retrieved from 103 uterine CS patients undergoing primary surgical treatment.

Medical records were abstracted including date of diagnosis, stage, age, race/ethnicity, body mass index in kg/m² (BMI), adjuvant therapy (chemotherapy, radiation therapy, or both), disease recurrence or progression, and date of death. Entry date for this analysis was defined as the date of histopathological diagnosis. The date of death was obtained by review of medical records and review of the Social Security Death Index.

Immunohistochemistry

Immunohistochemistry was performed on FFPE tissue sections (one case per glass slide) using a rabbit polyclonal antibody directed at the *IGF2* ligand (AB9574; Abcam Inc., Cambridge, MA). The optimized protocol has been previously validated and described [17]. In brief, Target Retrieval Solution, Citrate pH 6 (Dako North America, Inc.) was used for antigen retrieval, TBS containing 5% goat serum and 2% bovine serum albumin was used for blocking, and the primary antibody was used at a 1:100 dilution with an incubation of 1 h at room temperature. Secondary antibody and detection were performed using the Dako Envision+ Polymer System (Dako North America), followed by counterstaining with hematoxylin. Staining of all tissue slides was performed concurrently with staining of positive and negative control sections.

Representative-stained carcinosarcoma tissue sections were photographed on a Zeiss Axioskop II, and images shown in Figure 1 depict the TIFF image files without modifications, other than cropping of the size. The study pathologist (Y.W.), who was blinded to all clinical data, evaluated the *IGF2* staining intensity (0 negative, 1+ weak, 2+ moderate, 3+ strong) and the percentage of tumor cells with positive staining (1–100%) in each tumor compartment (epithelial cytoplasmic, epithelial nuclear, stromal cytoplasmic, and stromal nuclear). *IGF2* expression was summarized using the H-score, which is defined as the product of the staining intensity and the percentage of positive staining cells.

Statistical analysis

Patient demographics and clinical characteristics were described as appropriate for continuous and categorical variables. Standard descriptive statistics were used to describe H-scores. H-scores were dichotomized at their medians for each tumor compartment and categorized as low *IGF2* expression if the H-score was below the compartment median or high *IGF2* expression if the H-score was equal to or above the compartment median. Progression-free (PFS) and overall survival (OS) were

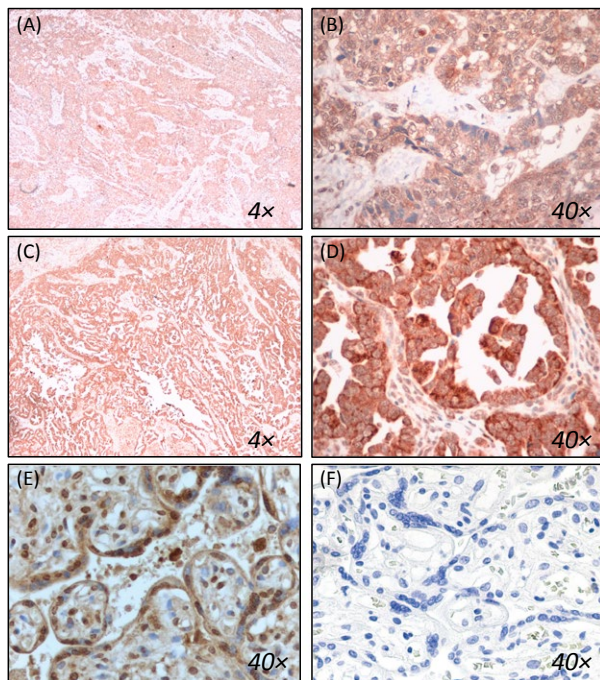


Figure 1. IGF2 immunohistochemical staining in uterine carcinosarcoma. Representative images of IGF2 immunohistochemistry are shown. In A (4x obj.) and B (40x obj.), the uterine carcinosarcoma tissue shows low IGF2 expression in all of the tumor compartments (EN, EC, SN, and SC). In contrast, the UCS tissue shown in C and D had high IGF2 expression in the EC and SC, but absent IGF2 expression in both nuclear compartments (EN and SN). Positive and negative control tissue sections are shown in E and F.

estimated using the Kaplan–Meier method and low-versus high IGF2 expression was compared using the log rank test. Univariable and multivariable Cox proportional hazard regression models were fit to examine the relationship of demographic variables and IGF2 expression to PFS and OS. Variables with $P < 0.10$ in the univariable analysis were included in the multivariable model. All P -values are two-sided, and a P -value of <0.05 was considered statistically significant. All analyses were performed using *Stata v13.0* (StataCorp, College Station, TX).

Results

Clinicopathologic characteristics of the 103 patients who underwent primary surgical resection for uterine carcinosarcoma are shown in Table 1. Reflecting the known epidemiological risk factors for carcinosarcoma, patients were generally older, overweight or obese, and predominantly black.

Approximately half of patients had early stage (FIGO Stage I or II) and half had advanced stage (FIGO Stage

Table 1. Demographic, pathologic, and treatment characteristics ($n = 103$).

Age (years)*	66.6 (11.2)	Adjuvant therapy	
BMI (kg/m ²)*	34.6 (10.4)	No	47 (45.6)
BMI Categories		Yes	56 (54.4)
<18.5	1 (1.0)	Adjuvant chemotherapy	
18.5–24.9	12 (11.7)	No	66 (64.1)
25.0–29.9	19 (18.4)	Yes	37 (35.9)
30.0–34.9	17 (16.5)	Adjuvant radiotherapy	
35.0–39.9	13 (12.6)	No	83 (80.6)
≥40.0	23 (22.3)	Yes	20 (19.4)
Missing	18 (17.5)	Type of adjuvant RT	
Race/ethnicity		EBRT	9 (45.0)
White	36 (35.0)	EBRT +	7 (35.0)
		brachytherapy	
Black	66 (64.1)	Brachytherapy	3 (15.0)
		only	
Other	1 (0.9)	Other	1 (5.0)
FIGO Stage		IGF2 H-scores [†]	
I	42 (40.8)	Epithelial	65 [0–285]
		nuclear	
II	12 (11.6)	Epithelial	180 [60–300]
		cytoplasmic	
III	30 (29.1)	Stromal	60 [0–200]
		nuclear	
IV	19 (18.5)	Stromal	160 [20–300]
		cytoplasmic	
Mesenchymal elements			
Homologous	47 (45.6)		
Heterologous	45 (43.7)		
Missing	11 (10.7)		
Residual tumor			
No	82 (79.6)		
Yes	20 (19.5)		
N/A	1 (0.9)		

*Reported as mean (standard deviation).

[†]Reported as median [Range].

III or IV) UCS. Most patients received adjuvant therapy following their surgery.

The median H-score for IGF2 staining was higher in the cytoplasmic compartment compared to the nuclear compartment in both the malignant epithelial and malignant stromal tissue (Table 1). In less than 20% of cases, either malignant epithelium or stroma was absent from the stained section; therefore, missing H-scores by compartment were as follows: epithelial nuclear and cytoplasmic $n = 9$, and stromal nuclear and cytoplasmic $n = 10$. Representative images of IGF2 immunohistochemical staining are shown in Figure 1. Histograms depicting the H-score distribution in each tissue component are shown in Figure S1. As the H-scores were not normally distributed, the median values rather than the mean values were selected as the optimal cutoff for defining high- versus low IGF2 expression. A moderate positive correlation was observed between IGF2 EN and IGF2 SN (Spearman's $\rho = 0.65$,

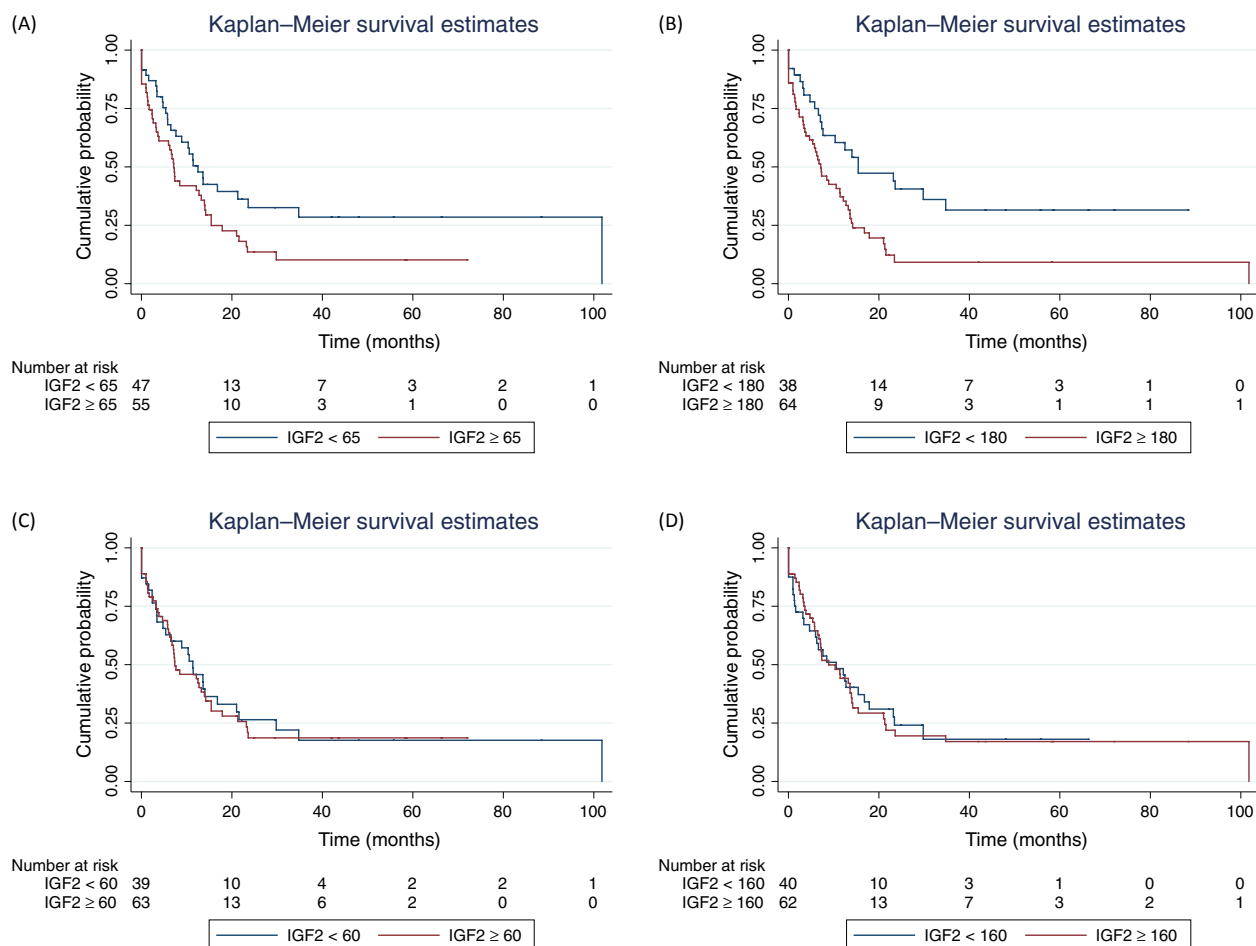


Figure 2. IGF2 expression and progression-free survival (PFS). Kaplan-Meier curves depict the PFS in patients with high IGF2 expression (red line) versus low IGF2 expression (blue line), measured in the following malignant tissue compartments: epithelial nuclear (A), epithelial cytoplasmic (B), stromal nuclear (C), and stromal cytoplasmic (D). Patients with high-CS epithelial IGF2 expression had reduced PFS compared with patients with low epithelial IGF2 expression (Log rank test $P = 0.036$ and $P = 0.002$ for epithelial nuclear and cytoplasmic compartments, respectively). Stromal IGF2 expression was not associated with PFS (Log rank test $P = 0.79$ and $P = 0.91$ for nuclear and cytoplasmic compartments, respectively).

$P < 0.05$), and between IGF2 EC and IGF2 SC (Spearman's $\rho = 0.39$, $P < 0.05$).

Progression-free survival

Over the course of follow-up (median 11.4 months), 75 patients had recurrence or progression of disease. The median PFS was 10.3 months [IQ Range: 3.2–23.2 months]. Progression-free survival curves by tissue/cellular CS compartment are depicted in Figure 2. Patients with high-CS epithelial IGF2 expression had reduced PFS compared with patients with low epithelial IGF2 expression (Log rank test $P = 0.036$ and $P = 0.002$ for epithelial nuclear and cytoplasmic compartments, respectively). Malignant stromal IGF2 expression was not associated with PFS (Log rank test $P = 0.79$ and $P = 0.91$ for stromal nuclear and cytoplasmic compartments, respectively).

The univariable and multivariable Cox proportional hazard's regression models for PFS are shown in Table 2. In univariable regression, black race, higher stage, high epithelial nuclear and cytoplasmic IGF2 expression were associated with an increased hazard of disease progression. Any adjuvant therapy was associated with a decreased hazard of progression of disease. In the multivariable model, after controlling for race, stage and adjuvant therapy (specifically chemotherapy), high IGF2 expression in both the epithelial nuclear and cytoplasmic compartments was associated with roughly twice the hazard of progression compared with low IGF2 expression (Epithelial nuclear HR = 1.80, 95% CI: 1.08–2.99, $P = 0.02$ and epithelial cytoplasmic HR = 2.07, 95% CI: 1.19–3.60, $P = 0.01$). After adjustment for IGF2 and stage, neither race, nor adjuvant therapy or chemotherapy, was independently associated with PFS.

Table 2. Univariable and multivariable Cox Proportional hazards models.

Variables	Progression-free survival		Overall survival	
	Univariable Hazard Ratio (95% CI, <i>P</i> -value)	Multivariable Hazard Ratio (95% CI, <i>P</i> -value)	Univariable Hazard Ratio (95% CI, <i>P</i> -value)	Multivariable Hazard Ratio (95% CI, <i>P</i> -value)
Age (years)	1.01 (0.99–1.03, 0.60)		1.02 (0.99–1.04, 0.10)	
BMI (kg/m ²)	0.99 (0.97–1.02, 0.56)		0.98 (0.95–1.01, 0.22)	
Race				
White	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Black	1.79 (1.06–3.02, 0.03)	1.68 (0.97–2.93, 0.07)	1.78 (1.04–3.06, 0.04)	1.68 (0.94–3.00, 0.08)
FIGO Stage				
I/II	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
III/IV	2.27 (1.41–3.65, <0.01)	2.11 (1.29–3.47, <0.01)	2.25 (1.39–3.67, <0.01)	2.28 (1.35–3.87, <0.01)
Sarcoma type				
Homologous	1.00 (reference)		1.00 (reference)	
Heterologous	1.10 (0.68–1.79, 0.70)		0.95 (0.57–1.58, 0.84)	
Adjuvant therapy - any				
No	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Yes	0.59 (0.37–0.94, 0.03)	0.55 (0.29–1.06, 0.07)	0.50 (0.31–0.80, <0.01)	0.40 (0.20–0.81, 0.01)
Adjuvant chemotherapy				
No	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Yes	0.64 (0.39–1.05, 0.08)	1.15 (0.58–2.28, 0.70)	0.59 (0.35–0.98, 0.04)	1.15 (0.55–2.41, 0.71)
Adjuvant radiation				
No	1.00 (reference)		1.00 (reference)	
Yes	0.83 (0.47–1.46, 0.51)		0.67 (0.37–1.24, 0.20)	
Epithelial nuclear IGF2				
H-score < median (65)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
H-score ≥ median (65)	1.64 (1.02–2.64, 0.04)	1.80 (1.08–2.99, 0.02)	1.72 (1.05–2.79, 0.03)	1.86 (1.10–3.15, 0.02)
Epithelial cytoplasmic IGF2				
H-score < median (180)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
H-score ≥ median (180)	2.17 (1.30–3.62, <0.01)	2.07 (1.19–3.60, 0.01)	2.11 (1.25–3.57, <0.01)	1.98 (1.11–3.54, 0.02)
Stromal nuclear IGF2				
H-score < median (60)	1.00 (reference)		1.00 (reference)	
H-score ≥ median (60)	1.07 (0.66–1.71, 0.79)		1.04 (0.64–1.70, 0.86)	
Stromal cytoplasmic IGF2				
H-score < median (160)	1.00 (reference)		1.00 (reference)	
H-score ≥ median (160)	1.03 (0.64–1.65, 0.91)		1.07 (0.66–1.75, 0.76)	

Overall survival

Seventy patients died of disease by the end of follow-up. The median OS was 13.9 months [IQ range: 6.23–51.9 months]. Overall survival curves by tissue/cellular CS compartment are depicted in Figure 3. Similar to PFS, OS was significantly lower in women with high-CS epithelial IGF2 expression (Log rank test $P = 0.03$ and $P = 0.005$ for epithelial nuclear and cytoplasmic compartments, respectively). Malignant stromal IGF2 expression was not associated with OS (Log rank test $P = 0.86$ and $P = 0.76$ for stromal nuclear and cytoplasmic compartments, respectively).

The univariable and multivariable Cox proportional hazard's regression models for OS are shown in Table 2. In univariable regression models, black race, higher stage, and high epithelial nuclear and cytoplasmic IGF2 expression were associated with an increased risk of death. Any adjuvant therapy was associated with a decreased risk of

death. In the multivariable model, after controlling for race, stage and adjuvant therapy, high IGF2 expression in both the epithelial nuclear and cytoplasmic compartments was associated with roughly twice the risk of death compared with low IGF2 expression (Epithelial nuclear HR = 1.86, 95% CI: 1.10–3.15, $P = 0.02$ and epithelial cytoplasmic HR = 1.98, 95% CI: 1.11–3.54, $P = 0.02$). Neither race nor adjuvant chemotherapy was independently associated with OS, after adjustment for IGF2 and stage.

Stage and race

After stratifying patients by FIGO stage, high epithelial cytoplasmic expression of IGF2 in early-stage UCS (stage I/II) was associated with an increased risk of disease progression (HR = 2.18, 95% CI: 1.05–4.54, $P = 0.04$) and death (HR = 3.41, 95% CI: 1.47–7.90, $P < 0.01$) after adjusting for race and adjuvant therapy (Table 3).

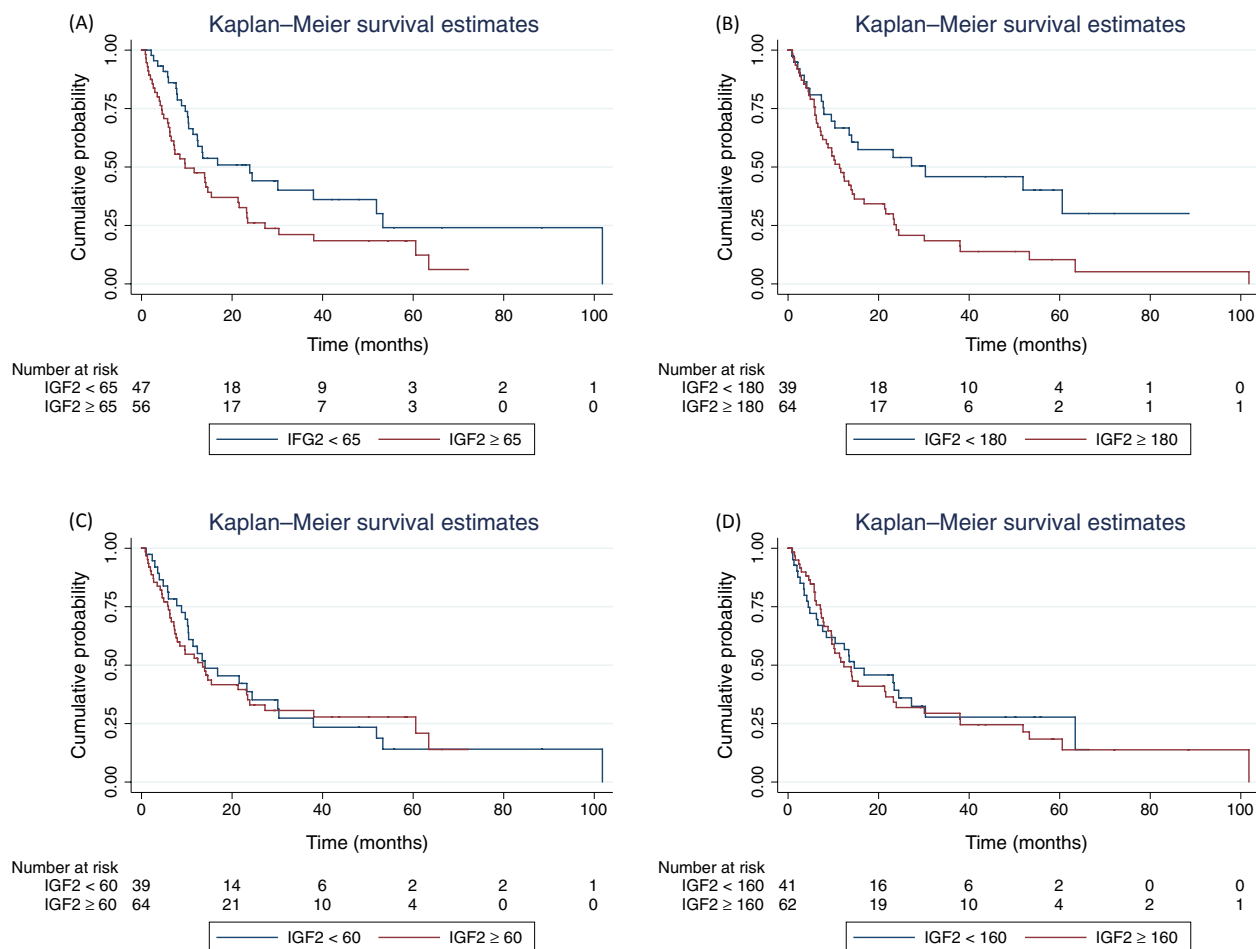


Figure 3. IGF2 expression and overall survival (OS). Kaplan-Meier curves depict the OS for patients with high IGF2 expression (red line) versus low IGF2 expression (blue line), as measured in the following malignant tissue compartments: epithelial nuclear (A), epithelial cytoplasmic (B), stromal nuclear (C), and stromal cytoplasmic (D). OS was significantly lower in women with high-CS epithelial IGF2 expression (Log rank test $P = 0.03$ and $P = 0.005$ for epithelial nuclear and cytoplasmic compartments, respectively). Stromal IGF2 expression was not associated with OS (Log rank test $P = 0.86$ and $P = 0.76$ for stromal nuclear and cytoplasmic compartments, respectively).

Additionally, in women with early-stage disease, black race was an independent poor prognostic factor for progression of disease (HR: 2.83, 95% CI: 1.21–6.64, $P = 0.02$) and death (HR = 2.80, 95% CI: 1.06–7.39; $P = 0.04$). In patients with Stage III/IV disease, neither race nor IGF2 expression significantly impacted PFS or OS.

In FIGO stage I/II disease, IGF2 expression was higher in the EC compartment of those with black race (Median H-score = 180, IQR: 160–190) compared to white race (Median H-score = 160, IQR: 80–180), and this was significantly different (Wilcoxon Rank Sum test, $P = 0.02$). Median H-scores did not differ between racial groups for the other tissue/cellular compartments (EN, or either stromal compartment).

After stratification of patients by race, high IGF2 in the EC or the EN compartments was independently associated with worse survival in black women with UCS. A

multivariable analysis was performed, including variables with $P < 0.1$ on univariable analysis (Table S1). Adjusting for stage and adjuvant therapy, the race-stratified HR for black women was 2.43 (95% CI: 1.18–5.01, $P = 0.02$) for high IGF2 in the EC, and the HR was 2.34 for high IGF2 in the EN (95% CI: 1.25–4.39, $P = 0.008$), as shown in Table 4.

Discussion

In this study, we found that elevated IGF2 protein expression is an independent poor prognostic factor in UCS. For patients whose tumor epithelial compartment demonstrated IGF2 expression equal to or above the median for the cohort, the risk of disease progression or death was approximately doubled. We also found that black women had higher tumor epithelial IGF2 expression

Table 3. Multivariable Cox proportional hazards models stratified by FIGO stage.

Variables	Stage I/II		Stage III/IV	
	PFS hazard ratio (95% CI, <i>P</i> -value)	OS hazard ratio (95% CI, <i>P</i> -value)	PFS hazard ratio (95% CI, <i>P</i> -value)	OS hazard ratio (95% CI, <i>P</i> -value)
Race				
White	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Black	2.83 (1.21–6.64, 0.02)	2.80 (1.06–7.39, 0.04)	0.96 (0.42–2.18, 0.92)	1.20 (0.53–2.74, 0.66)
Adjuvant therapy - any				
No	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Yes	0.52 (0.19–1.43, 0.21)	0.37 (0.12–1.19, 0.10)	0.45 (0.17–1.20, 0.11)	0.28 (0.10–0.76, 0.01)
Adjuvant chemotherapy				
No	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Yes	0.77 (0.25–2.36, 0.65)	0.64 (0.18–2.32, 0.50)	1.86 (0.73–4.74, 0.19)	1.77 (0.66–4.74, 0.26)
Epithelial Nuclear IGF2				
H-score < median (65)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
H-score ≥ median (65)	1.74 (0.78–3.86, 0.18)	1.50 (0.64–3.53, 0.36)	1.45 (0.69–3.03, 0.32)	1.81 (0.84–3.90, 0.13)
Epithelial Cytoplasmic IGF2				
H-score < median (180)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
H-score ≥ median (180)	2.18 (1.05–4.54, 0.04)	3.41 (1.47–7.90, <0.01)	2.11 (0.88–5.07, 0.10)	1.10 (0.48–2.52, 0.81)

Table 4. Cox stratified by race: Multivariable (keeping *P* < 0.10)

Variable	White			Black		
	Hazard Ratio	95% CI	<i>P</i> -value	Hazard Ratio	95% CI	<i>P</i> -value
Stage						
I/II	1.00 (ref.)		<0.001	1.00 (ref.)		0.04
III/IV	8.58	2.59–28.42		1.95	1.04–3.66	
Adjuvant therapy - Any						
No	1.00 (ref.)		0.001	1.00 (ref.)		0.02
Yes	0.15	0.05–0.47		0.48	0.27–0.87	
Epithelial Nuclear IGF2						
H-Score <median (65)				1.00 (ref.)		0.008
H-Score >median (65)				2.34	1.25–4.39	
Epithelial Cytoplasmic IGF2						
H-Score <median (180)				1.00 (ref.)		0.02
H-Score >median (180)				2.43	1.18–5.01	

compared to white women, and that high IGF2 was independently associated with worse survival in black women. Conversely, the impact of race on PFS and OS was attenuated, and no longer significant, after adjustment for IGF2 and stage. These novel findings suggest that tumor epithelial IGF2 expression is not only a prognostic biomarker in UCS, but also that IGF2 expression differences may contribute to the racial disparity in disease outcome in patients with UCS.

Unlike tumor epithelial IGF2 expression, the malignant stromal IGF2 expression was not prognostic of disease progression or survival. Recent studies of UCS show a high level of clonality, favoring a metaplastic origin of the sarcomatous elements derived from a common epithelial precursor as the carcinomatous elements [2, 5]. Interestingly, metastatic implants of UCS are composed of purely carcinomatous elements in the majority of cases

(69%), while 25% of cases have biphasic metastases and only 4% of cases have purely sarcomatous metastases [20]. These observations support the primacy of the carcinomatous component as the driver of the UCS phenotype, consistent with the findings of the present study.

The racial disparity in survival observed in this study is consistent with a prior analysis of a clinical UCS cohort, a subset of which comprises the IGF2 study cohort. As previously reported in a retrospective review of 158 patients treated for UCS at a single institution, outcomes were worse in black women compared to white women with early-stage disease after adjustment for adjuvant therapy [7].

Uterine corpus cancer is among the cancers with the largest racial disparity in survival. The five-year survival rate is 86% in white women diagnosed with uterine cancer compared with 66% in black women. In multiple other

cancers, including prostate, breast, and colorectal cancer, higher death rates are observed in blacks compared with whites in the United States [21]. Socioeconomic status and discrimination significantly contribute to racial disparity in cancer survival, including endometrial cancer outcomes [22]. Aggressive histologic types of endometrial cancer such as UCS occur more frequently in blacks, implicating tumor biology as a contributing factor to differences in mortality rates [23].

As in endometrial cancer, the aggressive types of breast cancer are more frequent in black women. A recent analysis of The Cancer Genome Atlas (TCGA) breast cancer cohort estimated that inherited germline variants account for 44% of racial differences in breast cancer subtype distribution [24]. Comparison of blacks and whites defined by genomically determined genetic ancestry identified not only differentially expressed genes and proteins, but also significantly altered DNA methylation sites in breast cancer tissue. However, an earlier study of endometrial cancers from black and white women did not reveal significant differences in gene transcription, but was limited by the small number of patients [25]. Thus, nonbiological and biological factors likely contribute to racial disparity in uterine cancer survival.

While race is a social construct, self-identified race moderately correlates with genetic ancestry. It has been reported that metabolic variables such as insulin resistance preferentially cluster with genetic ancestry [26]. The tumor necrosis factor (TNF)- α axis, a cytokine initiator of inflammation, shows differences in genotype and allele frequencies (in TNF- α and its receptor genes TNFR1 and TNFR2) between blacks and whites [27]. Interestingly, TNF- α has been identified as a regulator of IGF2 production by adipocytes [28].

Nonbiological factors may result in identifiable changes in downstream biological mediators, wherein stress-inducing conditions, nutrition, and environmental toxins alter epigenetic markers and contribute to health disparities [29]. For example, increasing poverty and nonwhite race is associated with elevated C-reactive protein (CRP) levels [30]. Of note, IGF2 is a regulator of CRP, and IGF2 was among the first genes identified to be altered in expression as a result of fetal exposure to poor nutrition [31]. In addition, oxidative stress induces IGF2 overexpression via loss of imprinting triggered by NF- κ B activation [32].

IGF2 is one of a relatively small number of imprinted genes in mammals, by which epigenetic silencing results in monoallelic expression specific to parental origin [10]. Most imprinted genes have key functions during embryonic development as is the case for *IGF2*. Upregulation of *IGF2* occurs in both childhood and adult malignancies, and its overexpression is linked to an aggressive phenotype of ovarian and prostate cancer [17, 33]. Several important

IGF2 regulatory mechanisms have been identified, many of which overlap with functions during fetal development, including altered transcription factor expression, epigenetic changes such as altered DNA methylation, as well as changes in post-transcriptional and post-translational modulators of IGF2 expression [10].

Epigenetic events, particularly DNA methylation changes, are triggered by environmental factors and are key drivers of cancer development and progression [34]. Differences in methylation status have been previously linked to racial disparity in breast, prostate, colorectal, and endometrial cancers [35]. The increased tumor expression of IGF2 observed in black women with UCS may reflect differences in DNA methylation at its regulatory sites. We speculate that IGF2 expression is a potential biomarker of prior environmental exposures and stressors as well as heritable variations in metabolic and inflammatory pathways which interact to regulate IGF2 and cancer mortality in UCS.

Our group has previously shown that increased tumor expression of IGF2 in epithelial ovarian cancer cells is associated with resistance to paclitaxel and other microtubule stabilizing drugs, and that IGF-targeting therapy restored drug sensitivity in cell line and xenograft models of ovarian cancer [17, 36]. Other groups have shown that IGF2 overexpression is associated with resistance to chemotherapy agents such as cisplatin, adriamycin, and fluorouracil [37–39]. As paclitaxel and platinum drugs comprise the most commonly used adjuvant treatment for UCS, modulation of chemosensitivity by IGF2 is highly clinically relevant. Our laboratory is presently evaluating the ability of IGF2-targeting therapy to enhance efficacy of chemotherapy using primary UCS cell lines and patient-derived UCS xenografts.

In summary, we have identified IGF2 protein expression as an independent poor prognostic biomarker in UCS, a lethal uterine cancer that disproportionately impacts black women. These novel findings, in conjunction with prior studies of IGF2 in cancer, further substantiate that IGF2 is a biological mediator of an aggressive cancer phenotype and a potential therapeutic target. Moreover, we have uncovered a novel potential association of IGF2 with racial disparity in UCS, which merits further investigation.

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Conflicts of Interest

The authors declare that there are no conflict of interests.

References

1. Siegel, R. L., K. D. Miller, and A. Jemal. 2016. Cancer statistics, 2016. *CA Cancer J. Clin.* 66:7–30.
2. Zhao, S., S. Bellone, S. Lopez, D. Thakral, C. Schwab, D. P. English, et al. 2016. Mutational landscape of uterine and ovarian carcinosarcomas implicates histone genes in epithelial- mesenchymal transition. *Proc. Natl Acad. Sci. USA* 113:12238–12243.
3. Jones, S., N. Stransky, C. L. McCord, E. Cerami, J. Lagowski, D. Kelly, et al. 2014. Genomic analyses of gynaecologic carcinosarcomas reveal frequent mutations in chromatin remodelling genes. *Nat. Commun.* 5:5006.
4. Somarelli, J. A., M.-K. Boss, J. I. Epstein, A. J. Armstrong, and M. A. Garcia-Blanco. 2015. Carcinosarcomas: tumors in transition? *Histol. Histopathol.* 30:673–687.
5. Cherniack, A. D., H. Shen, V. Walter, C. Stewart, B. A. Murray, R. Bowlby, et al. 2017. Integrated molecular characterization of uterine carcinosarcoma. *Cancer Cell* 31:411–423.
6. Brooks, S. E., M. Zhan, T. Cote, and C. R. Baquet. 2004. Surveillance, epidemiology, and end results analysis of 2677 cases of uterine sarcoma 1989-1999. *Gynecol. Oncol.* 93:204–208.
7. Erickson, B. K., D. W. Doo, B. Zhang, W. K. Huh, and C. A. Leath. 2014. Black race independently predicts worse survival in uterine carcinosarcoma. *Gynecol. Oncol.* 133:238–241.
8. Seagle, B.-L. L., M. Kanis, M. Kocherginsky, J. B. Strauss, and S. Shahabi. 2017. Stage I uterine carcinosarcoma: matched cohort analyses for lymphadenectomy, chemotherapy, and brachytherapy. *Gynecol. Oncol.* 145:71–77.
9. Vorgias, G., and S. Fotiou. 2010. The role of lymphadenectomy in uterine carcinosarcomas (malignant mixed mullerian tumours): a critical literature review. *Arch. Gynecol. Obstet.* 282:659–664.
10. Brouwer-Visser, J., and G. S. Huang. 2015. IGF2 signaling and regulation in cancer. *Cytokine Growth Factor Rev.* 26:371–377.
11. Lui, J. C., and J. Baron. 2013. Evidence that IGF2 down-regulation in postnatal tissues and up-regulation in malignancies is driven by transcription factor E2f3. *Proc. Natl Acad. Sci. USA* 110:6181–6186.
12. Rikhof, B., S. de Jong, A. J. H. Suurmeijer, C. Meijer, and W. T. A. van der Graaf. 2009. The insulin-like growth factor system and sarcomas. *J. Pathol.* 217:469–482.
13. Roy, R. N., A. H. Gerulath, A. Cecutti, and B. R. Bhavnani. 2000. Loss of IGF-II imprinting in endometrial tumors: overexpression in carcinosarcoma. *Cancer Lett.* 153:67–73.
14. Honma, H., Y. Takahashi, M. Matsui, T. Satoh, I. Fukuda, K. Takahashi, et al. 2015. Non-islet cell tumor hypoglycemia is caused by big IGF-II in a patient with a carcinosarcoma of the uterus. *Intern. Med.* 54:3165–3169.
15. Li, H., I. S. Batth, X. Qu, L. Xu, N. Song, R. Wang, et al. 2017. IGF-IR signaling in epithelial to mesenchymal transition and targeting IGF-IR therapy: overview and new insights. *Mol. Cancer.* 16:6.
16. Pollak, M. 2012. The insulin and insulin-like growth factor receptor family in neoplasia: an update. *Nat. Rev. Cancer* 12:159–169.
17. Huang, G. S., J. Brouwer-Visser, M. J. Ramirez, C. H. Kim, T. M. Hebert, J. Lin, et al. 2010. Insulin-like growth factor 2 expression modulates Taxol resistance and is a candidate biomarker for reduced disease-free survival in ovarian cancer. *Clin. Cancer Res.* 16:2999–3010.
18. Lu, L., D. Katsaros, A. Wiley, I. A. Rigault de la Longrais, M. Puopolo, P. Schwartz, et al. 2006. Promoter-specific transcription of insulin-like growth factor-II in epithelial ovarian cancer. *Gynecol. Oncol.* 103:990–995.
19. Lu, L., D. Katsaros, A. Wiley, I. A. Rigault de la Longrais, H. A. Risch, M. Puopolo, et al. 2006. The relationship of insulin-like growth factor-II, insulin-like growth factor binding protein-3, and estrogen receptor- α expression to disease progression in epithelial ovarian cancer. *Clin. Cancer Res.* 12:1208–1214.
20. Sreenan, J. J., and W. R. Hart. 1995. Carcinosarcomas of the female genital tract. A pathologic study of 29 metastatic tumors: further evidence for the dominant role of the epithelial component and the conversion theory of histogenesis. *Am. J. Surg. Pathol.* 19:666–674.
21. DeSantis, C. E., R. L. Siegel, A. G. Sauer, K. D. Miller, S. A. Fedewa, K. Alcaraz, et al. 2016. Cancer statistics for African Americans, 2016: Progress and opportunities in reducing racial disparities. *CA Cancer J. Clin.* 66:290–308.
22. Fader, A. N., E. B. Habermann, K. T. Hanson, J. F. Lin, E. C. Grendys, and S. C. Dowdy. 2016. Disparities in treatment and survival for women with endometrial

- cancer: a contemporary national cancer database registry analysis. *Gynecol. Oncol.* 143:98–104.
23. Smotkin, D., N. S. Nevadunsky, K. Harris, M. H. Einstein, Y. Yu, and G. L. Goldberg. 2012. Histopathologic differences account for racial disparity in uterine cancer survival. *Gynecol. Oncol.* 127:616–619.
 24. Huo, D., H. Hu, S. K. Rhie, E. R. Gamazon, A. D. Cherniack, J. Liu, et al. 2017. Comparison of breast cancer molecular features and survival by African and European ancestry in the cancer genome atlas. *JAMA Oncol.* 3:1654–1662.
 25. Ferguson, S. E., A. B. Olshen, D. A. Levine, A. Viale, R. R. Barakat, and J. Boyd. 2006. Molecular profiling of endometrial cancers from African-American and Caucasian women. *Gynecol. Oncol.* 101:209–213.
 26. Louwers, Y. V., O. Lao, B. C. J. M. Fauser, M. Kayser, and J. S. E. Laven. 2014. The impact of self-reported ethnicity versus genetic ancestry on phenotypic characteristics of polycystic ovary syndrome (PCOS). *J. Clin. Endocrinol. Metab.* 99:E2107–E2116.
 27. Menon, R., D. R. Velez, N. Morgan, S. J. Lombardi, S. J. Fortunato, and S. M. Williams. 2008. Genetic regulation of amniotic fluid TNF-alpha and soluble TNF receptor concentrations affected by race and preterm birth. *Hum. Genet.* 124:243–253.
 28. Gude, M. F., J. Frystyk, A. Flyvbjerg, J. M. Bruun, B. Richelsen, and S. B. Pedersen. 2012. The production and regulation of IGF and IGF-BPs in human adipose tissue cultures. *Growth Horm. IGF Res.* 22:200–205.
 29. Thayer, Z. M., and C. W. Kuzawa. 2011. Biological memories of past environments: epigenetic pathways to health disparities. *Epigenetics* 6:798–803.
 30. Nazmi, A., and C. G. Victora. 2007. Socioeconomic and racial/ethnic differentials of C-reactive protein levels: a systematic review of population-based studies. *BMC Public Health* 7:212.
 31. Li, S.-F., Y.-W. Hu, J.-Y. Zhao, X. Ma, S. G. Wu, J. B. Lu, et al. 2015. Ox-LDL upregulates CRP expression through the IGF2 pathway in THP-1 macrophages. *Inflammation* 38:576–583.
 32. Yang, B., J. Wagner, N. Damaschke, T. Yao, S. M. Wuerzberger-Davis, M. H. Lee, et al. 2014. A novel pathway links oxidative stress to loss of insulin growth factor-2 (IGF2) imprinting through NF- κ B activation. *PLoS ONE* 9:e88052.
 33. Vidal, S. J., V. Rodriguez-Bravo, S. A. Quinn, R. Rodriguez-Barrueco, A. Lujambio, E. Williams, et al. 2015. A targetable GATA2-IGF2 axis confers aggressiveness in lethal prostate cancer. *Cancer Cell* 27:223–239.
 34. Herceg, Z., and T. Vaissière. 2011. Epigenetic mechanisms and cancer: an interface between the environment and the genome. *Epigenetics* 6:804–819.
 35. Ahmad, A., S. Azim, H. Zubair, M. A. Khan, S. Singh, J. E. Carter, et al. 2017. Epigenetic basis of cancer health disparities: Looking beyond genetic differences. *Biochim. Biophys. Acta* 1868:16–28.
 36. Brouwer-Visser, J., J. Lee, K. McCullagh, M. J. Cossio, Y. Wang, and G. S. Huang. 2014. Insulin-like growth factor 2 silencing restores taxol sensitivity in drug resistant ovarian cancer. *PLoS ONE* 9:e100165.
 37. Li, B., S. W. Tsao, K. W. Chan, D. L. Ludwig, R. Novosyadlyy, Y. Y. Li, et al. 2014. Id1-induced IGF-II and its autocrine/endocrine promotion of esophageal cancer progression and chemoresistance—implications for IGF-II and IGF-IR-targeted therapy. *Clin. Cancer Res.* 20:2651–2662.
 38. Shimizu, T., E. Sugihara, S. Yamaguchi-Iwai, S. Tamaki, Y. Koyama, W. Kamel, et al. 2014. IGF2 preserves osteosarcoma cell survival by creating an autophagic state of dormancy that protects cells against chemotherapeutic stress. *Cancer Res.* 74:6531–6541.
 39. Ogawa, T., K. Ogawa, K. Shiga, T. Furukawa, H. Nagase, S. Hashimoto, et al. 2010. Upregulation of IGF2 is associated with an acquired resistance for cis-diamminedichloroplatinum in human head and neck squamous cell carcinoma. *Eur. Arch. Otorhinolaryngol.* 267:1599–1606.

Supporting Information

Additional supporting information may be found in the online version of this article:

Figure S1. Histograms for H-score distribution for each malignant tissue compartment.

Table S1. Cox stratified by Race: Univariable.