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Neratinib shows efficacy in the treatment of HER2 amplified carcinosarcoma *in vitro* and *in vivo*

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Abstract

Objective—Carcinosarcoma is a deadly gynecologic malignancy with few effective treatment options. The study of new therapies is difficult because of its rarity. The objective of this study was to determine the efficacy of neratinib in the treatment of HER2 amplified carcinosarcoma.

Methods—The efficacy of neratinib in the treatment of HER2 amplified carcinosarcoma was determined *in vitro* using seven primary carcinosarcoma cell lines with differential expression of HER2/neu. Data regarding IC₅₀, cell cycle distribution, and cell signaling changes were assessed by flow cytometry. The efficacy of neratinib was determined in treating mice harboring HER2 amplified carcinosarcoma xenografts.

Results—Two of seven (28.5%) carcinosarcoma cell lines were HER2/neu amplified. HER2/neu amplified cell lines SARARK6 and SARARK9 were significantly more sensitive to neratinib than the five non-HER2/neu amplified carcinosarcoma cell lines (mean±SEM IC₅₀: 0.014μM±0.004 vs. 0.164μM±0.019 *p*=0.0003). Neratinib treatment caused a significant build up in G0/G1 phase of the cell cycle, arrest auto phosphorylation of HER2/neu and activation of S6. Neratinib inhibited tumor growth (*p*=0.012) and prolonged survival in mice harboring HER2 amplified carcinosarcoma xenografts (*p*=0.0039).

Conclusions—Neratinib inhibits HER2 amplified carcinosarcoma proliferation, signaling, cell cycle progression and tumor growth *in vitro*. Neratinib inhibits HER2/neu amplified xenograft growth and improves overall survival. Clinical trials are warranted.

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Keywords

Carcinosarcoma; HER2/neu; TKI; Targeted therapy; ErbB2

Introduction

Carcinosarcoma is a rare and highly aggressive gynecologic malignancy accounting for less than 5% of all gynecologic cancers [1]. Carcinosarcomas can arise in the ovary, cervix or uterus. Previously published data from the SEER data base suggests that age adjusted rate of carcinosarcoma development in the uterus is 0.6/100,000 and in the ovary 0.19/100,000 [2]. Despite aggressive surgical and adjuvant therapy 5 year survival rates for uterine carcinosarcoma are approximately 60% for early (stage I/II) disease and 9–22% for more advanced disease [3]. Similarly, ovarian carcinosarcomas account for less than 4% of all newly diagnosed ovarian carcinomas and carry a significantly worse prognosis than their epithelial ovarian carcinoma counterparts [4]. Standard treatment for carcinosarcoma is aggressive surgical debulking followed by chemotherapy with or without radiation. The rarity of these tumors has made determination of the best adjuvant therapy difficult and completing large prospective randomized trials remains challenging. As a result a considerable amount of effort has been made to better understand the biology of the disease, its developmental origins as well as common genetic alterations and activated molecular pathways in an attempt to improve treatments and ultimately survival.

Carcinosarcomas are composed of an epithelial component as well as a sarcomatous component. The histology of the sarcomatous component is classified as either homologous or heterologous based on the presence of tissues that are either native or foreign to the uterus respectively. Many theories regarding the mechanism of development of this tumor, composed of two dissimilar cell populations, have been proposed [5]. Some studies regarding the origins of carcinosarcoma suggest that it develops as a result of epithelial to mesenchymal transformation [6] [7]. The dysregulation of a number of pathways commonly associated with high grade endometrial cancer including PIK3CA, PTEN, and KRAS have been suggested to influence EMT and maybe precursor molecular alterations prior to the development of carcinosarcoma [8]. These data suggest that the epithelial component of the carcinosarcoma drives the growth and proliferation of these rare and aggressive tumors.

Research is focusing on development of targeted therapies in the treatment of all cancers. The use of next generation sequencing will continue to evolve and elucidate new targets for potential drug development. Current targeted therapies are focused on highly active driver pathways found within tumors. The dysregulation of the ErbB family of tyrosine kinases has been implicated in the development of a number of tumors including breast, colorectal, anal, gastric, bladder, prostate, esophageal, head and neck, endometrial and non-small cell lung cancers [9]. It is well known that amplification or mutation of the ErbB pathways can lead to increased metabolic rate, proliferation and oncogenesis. Previously reported preclinical data in HER2/neu amplified uterine serous carcinoma suggests that targeting this pathway with the small tyrosine kinase inhibitor, neratinib, maybe an efficacious strategy in the treatment of highly aggressive gynecologic malignancies [10]. The amplification of HER2/neu has

been reported to occur in 20–25% of uterine carcinosarcomas while the frequency of amplification in ovarian carcinosarcomas has not yet been reported [11]. The frequency of amplification makes HER2/neu an attractive target for new molecularly targeted therapies.

The objective of this paper is to describe the effects of neratinib, an oral small tyrosine kinase inhibitor of ErbB1 and HER2/neu, on HER2/neu amplified carcinosarcoma proliferation, molecular driver pathway activation, and cell cycle distribution *in vitro* and its efficacy in the treatment of HER2 amplified xenografts in a mouse model.

Methods

Establishing cell lines

Prior to surgical staging, patients were consented for tumor banking. This was carried out through a Yale University institutional review board approved HIC, which was designed in accordance with the Helsinki Declaration. At the time of frozen section small portions of viable tumor were collected for processing and establishment of primary cell lines. Tumor samples were processed and deidentified as previously described [12]. Briefly tumors were processed using a scalpel for mechanical disruption in an enzymatic solution of 0.14% collagenase type 1 (Sigma) and 0.01% DNase (Sigma, 2000 KU/mg) in RPMI 1640. Dissociated tumor pieces were allowed to incubate in the same solution while stirring for one hour at room temperature. The samples were then washed with RPMI and plated in Petri dishes in Media (RPMI containing 10% FBS, 1% penicillin with streptomycin and 1% fungizone). They were kept in an incubator at 37 °C with 5% CO₂. Cell culture were continually checked and monitored for growth.

Determination of HER2 expression

Cell blocks were created and Immunohistochemistry (IHC) and fluorescence *in situ* hybridization (FISH) were carried out as previously described [12] [13]. In summary, HER2/neu IHC was carried out on cell blocks created from paraffin embedded pellets of established cell line cultures. After 5µm sections were deparafinized and rehydrated antibody to HER2/neu was applied at 1:800 (Thermo Fisher Scientific, Fremont, CA). The intensity of staining was then classified as 0 (no staining observed), 1+ (light staining weak staining in <10% of cells), 2+ (moderate staining-weak staining in 10% of cells and strong staining in less than 30% of cells), or 3+ (strong staining- Uniform intense staining in >30% of cells). Positive and negative controls were used as a comparison in each case. Following IHC, FISH analysis was performed using the PathVysion HER2 DNA-FISH-Kit (Abbott Molecular Inc., Abbott Park, IL, USA) according to the manufacturer's instructions and as previously described [13]. Fluorescent signals in at least 30 non-overlapping interphase intact nuclei were scored using a Zeiss Axioplan 2 microscope (Carl Zeiss Meditec, Inc., Dublin, CA, USA) with a 100x planar objective. Carcinosarcoma samples with fluorescent signals of HER2 to chromosome 17 ratio ≥ 2.2 were scored as amplified.

Drug

Neratinib (HKI-272) was obtained from Puma Biotechnology (Los Angeles, CA). It was diluted in DMSO at a concentration of 10mM. Serial dilutions of the drug were made using

DMSO to reach final concentrations of 1mM, 0.1mM, 0.05mM and 0.005mM. For in vivo dosing, drug was suspended in sterile water containing 0.5% methyl cellulose (Sigma life sciences, St. Louis, MO) at a concentration of 8 mg/ml.

Determination of IC₅₀

Data regarding the IC₅₀ of each cell line were gathered by first harvesting cells for each cell line during the log phase of growth. They were counted using a hemocytometer and plated in 6 well plates at a concentration of 20,000 cells/ml. Cells were allowed to establish over a 24 hour period and then treated with scalar quantities of neratinib ranging from 0.005 μ M to 0.750 μ M. After a 72 hour incubation period, cells were harvested in their entirety, stained with propidium iodide (Sigma life sciences, St. Louis, MO) (2 μ l of 500 μ g/ml stock solution in PBS with 1% azide and 2% fetal bovine serum) and read by flow cytometry. Data were then normalized to control wells and considered percent viable cells as a mean $\pm$ SEM. The IC₅₀ of each cell line was determined by comparing the log base 10 of drug concentration in each well to the percentage of viable cells using a non-parametric 3 parameter regression. These calculations were performed using prism 5 software (Graph Pad Prism Software Inc. San Diego, CA). All cell lines were tested in at least triplicate.

Changes in cell cycle distribution

Cells from representative cell lines were harvested in log phase of growth and plated at a concentration of 20,000 cells/ml in 6 well plates. They were exposed to neratinib at concentrations of 0.01 μ M, 0.065 μ M, or 0.133 μ M. After 48 hours of incubation, cells were collected in their entirety and were fixed by suspending in 70% ethanol for 30 minutes on ice. Each sample was washed with PBS and treated with 100 μ l of ribonuclease in PBS at a concentration of 100 μ g/ml at room temperature for 5 minutes. Propidium iodide (50 μ g/ml in PBS) was then added to each sample to reach a final volume of 500 μ l. Cell cycle was then analyzed by flow cytometry using BD Cell Quest Pro software. Data were analyzed using Flowjo.

Alterations in cell signaling

Cells from the HER2/neu amplified SARARK 6 carcinosarcoma cell line were harvested in the log phase of growth. They were plated at a concentration of 100,000 cells/ml in 6 well plates. They were allowed to incubate for 24 hours and treated with neratinib at 0.065 μ M. Cells were harvested at 2, 4, 6, 8 and 16 hours. Each sample fixed with 2% paraformaldehyde for 10 minutes, permeabilized with 90% methanol on ice for 30 minutes and blocked for 10 minutes in PBS containing 0.5% BSA (Sigma). Each sample was suspended in 100 μ l of 0.5% BSA and antibody to either p-HER2/neu-1221, p-S6 (Cell Signaling Technologies, Danvers, MA), or no primary antibody was added to each tube. Samples were allowed to incubate for one hour on ice. Samples were washed and stained with secondary fluorescein conjugated antibody (Millipore) for 20–30 minutes. Samples were then suspended in PBS and read by flow cytometry. The mean fluorescent intensity was then compared between treated and untreated control cells. Dose response assays were carried out in a similar fashion but 0.01 μ M, 0.065 μ M, and 0.133 μ M of neratinib were used to assess levels of both phosphorylated S6 and HER2/neu at 12 and 8 hours respectively. All data are expressed as mean $\pm$ standard deviation.

***In vivo* mouse model**

The HER2/neu amplified carcinosarcoma cell line SARARK 6 was expanded in 150ml flasks. Cells were harvested and counted using a hemocytometer. Cells were suspended in a 50%/50% mixture of PBS and matrigel (BD Biosciences). They were injected subcutaneously at a concentration of 7.2 million cells per SCID mouse (Harlan Netherlands, Horst, Netherlands). A total of 10 mice were used. Mice were divided into two groups, those treated with neratinib at 40mg/kg and those treated with vehicle (0.5% methylcellulose). Mice were treated by oral gavage once daily after tumors reached at least 0.5cm in one diameter. Mouse weights and tumor sizes were assessed at least twice weekly. Mice were sacrificed if tumors reached 1 cm³, if they appeared in poor health or if tumors were necrotic. Tumor volumes were calculated using the formula (width × width × height) × 0.5). All mice were housed and treated in accordance with the policies set forth by the Institutional Animal Care and Use Committee (IACUC) at Yale University.

Statistical analysis

Comparison of neratinib's efficacy was carried out between HER2 amplified and non-amplified uterine carcinosarcoma cell lines. The IC₅₀ values of the 6 cell lines were compared using a one way analysis of variance. Grouped mean IC₅₀ values were compared using two tailed student's t-test. Mean fluorescence intensities of phosphorylated S6 and HER2/neu as well as cell cycle data were compared between HER2 amplified and non-amplified cell lines using a one way analysis of variance or two tailed student's t-test. Overall survival in mice harboring HER2 amplified uterine carcinosarcoma xenografts, treated with neratinib or vehicle, was compared using a Kaplan Meier curve and log rank test. All statistical analysis was performed using Prism 5 software (Graph Pad Prism Software Inc. San Diego, CA). A *p* value of <0.05 was considered statistically significant.

Results

Determination of IC₅₀ of HER2 amplified and non-amplified carcinosarcoma

The characteristics of the cell lines tested and data regarding patient age, race and primary site of tumor development are presented in Table 1. Each of the 7 cell lines tested were established as long term cultures. They were plated in 6 well plates and treated with scalar concentrations of neratinib. After treatment they were allowed to incubate with the drug for 72 hours. The effect of neratinib on cell proliferation was determined through flow cytometric analysis and comparing treated to control wells. Of the currently available carcinosarcoma cell lines SARARK6 and SARARK 9 were the only cell lines that displayed amplification in the HER2/neu pathway. When comparing HER2/neu amplified ovarian carcinosarcoma cell line SARARK6 to the other two HER2/neu non-amplified ovarian carcinosarcoma cell lines the IC₅₀ values were significantly lower for SARARK6 *p*<0.0001. Similarly when comparing HER2/neu amplified uterine carcinosarcoma cell line SARARK9 to the other three HER2/neu non-amplified uterine carcinosarcoma cell lines the IC₅₀ values were significantly lower for SARARK9 *p*<0.0001 (Figure 1A). Comparisons of the overall mean IC₅₀ values for the HER2/neu amplified and non-amplified cell lines revealed a significant difference between the means of the two groups mean±SEM IC₅₀:0.014μM

± 0.004 vs. $0.164\mu\text{M} \pm 0.019$ $p=0.0003$ (Figure 1B). These data suggest that HER2/neu amplified carcinosarcoma are significantly more sensitive to the effects of neratinib *in vitro*.

Neratinib's effect on signaling through HER2/neu

The differential effect of neratinib on the proliferation of HER2/neu amplified compared to non-amplified carcinosarcoma suggests that the amplification of HER2/neu confers sensitivity to the cell line. In order to further substantiate this supposition, the effects of neratinib treatment on HER2/neu autophosphorylation and S6 activation were analyzed by flow cytometry. The HER2/neu amplified cell line SARARK 6 was plated in 6 well plates and allowed to incubate for 24 hours. Cells were then treated with nearly half (65nm) the physiologic concentration of neratinib attainable in humans (133nm) after treatment with a 240mg oral tablet [14]. Contents of the wells were harvested at various time points to determine the effects of neratinib on both HER2/neu autophosphorylation and S6 activation overtime. Data revealed that after treatment with neratinib the autophosphorylation of HER2/neu was significantly inhibited as early as 2 hours after treatment, $p=0.035$ (Figure 2A). The inhibition of HER2 autophosphorylation was durable and reached its peak at 8 hours, $p=0.027$ (Figure 2A). The downstream effects on the phosphorylation of S6 were slightly delayed, reaching significance at 6 hours, $p=0.031$ (Figure 2B) with maximal arrest of phosphorylation at 12 hours, $p=0.005$ (Figure 2B). Using these data, dose response studies were designed to assess the effects of both low and physiologic doses of neratinib on these signaling pathways.

Dose response data relating to changes in phosphorylation were gathered by plating SARARK6 in 6 well plates. Each well was subsequently treated with either 10nm or 133nm concentrations of neratinib. Cells were then allowed to incubate for either 8 hours or 12 hours to determine the effects on the phosphorylation of HER2/neu and S6 respectively. Flow cytometry was used to determine the effects of neratinib on phosphorylation and showed that both very low (10nm) and physiologically attainable concentrations of neratinib (133nm) significantly inhibited the autophosphorylation of HER2/neu ($p=0.0047$) and activation of S6 ($p=0.0001$), as shown in figures 3C and D respectively. These data suggest that neratinib is a robust and durable inhibitor of the HER2/neu driver pathway leading to arrest of activation of S6 both at low and physiologically relevant concentrations of neratinib.

Cell cycle analysis

Data regarding the effects of neratinib on HER2/neu amplified carcinosarcoma suggests that sensitivity is dependent on HER2 amplification and arrest of HER2 signaling leads to decreased activation of the transcription factor S6 and eventually cell death. Given these findings, data were gathered to determine the effect of neratinib on progression through the cell cycle. SARARK 6 was plated in 6 well plates and treated with varying concentrations of neratinib. After 48 hours of incubation, cells were collected and changes in cell cycle distribution were determined and compared to untreated cells. Results show that with neratinib treatment at both $0.065\mu\text{M}$ ($p=0.03$) and $0.133\mu\text{M}$ ($p=0.02$) there was significant buildup in the G0/G1 phase of the cell cycle (Figure 3). Arrest in the G0/G1 phase of the cell cycle likely leads to apoptosis and tumor death.

Neratinib's effect on HER2/neu amplified xenografts

Neratinib's effect *in vivo* was determined by establishing HER2/neu amplified xenografts of SARARK 6. When subcutaneous tumors reached at least 0.5 cm in a single dimension treatment was initiated. Of the 10 mice injected with SARARK6, a total of nine mice developed subcutaneous tumors. Of the nine mice available, 4 were considered vehicle and 5 were started on neratinib 40mg/kg 5 days weekly by oral gavage. Previous preclinical data in breast cancer using neratinib suggested a wide range of effective and safe dosages ranging from 10mg/kg/day to 80mg/kg/day, thus a mid-range dose of 40 mg/kg 5 days a week was selected [15]. At the start of treatment, tumor sizes were determined and there was no significant difference between the neratinib and vehicle groups ($p=0.2591$). Tumors were assessed at least twice weekly and mice were sacrificed if tumors were necrotic, reached 1 cm³, or mice appeared to be in poor health. Over the course of 40 days mice tolerated treatment with neratinib well and gained weight at a similar rate compared to vehicle treated controls (data not shown). Treatment with neratinib significantly inhibited tumor growth as early as 9 days after treatment began ($p=0.012$). Over the course of the treatment period neratinib significantly inhibited tumor growth with the greatest difference noted at 30 days ($p<0.0001$) as shown in figure 4A. Treatment with neratinib also significantly improved overall survival compared to vehicle treated mice, $p=0.0039$ (Figure 4B). These data suggest that neratinib is an efficacious treatment for HER2/neu amplified carcinosarcoma *in vivo*.

Discussion

Carcinosarcoma is a rare and aggressive gynecologic cancer composed of both an epithelial and sarcomatous component. The rarity, aggressive nature, and rapid development of chemotherapeutic resistance make these tumors difficult to cure and even more difficult to study potential treatments. The data presented in this study support the potential use of neratinib in the treatment of patients with HER2 amplified uterine or ovarian carcinosarcoma. Results show that neratinib effectively inhibits signaling through the HER2/neu pathway ultimately leading to less activation of S6. Arrest of transcription leads to build up in the G0/G1 phase of the cell cycle which in turn likely leads to apoptosis and cell death. The effects of neratinib *in vitro*, as demonstrated in this study are rapid and long lasting. *In vivo* data further support these conclusions with a significant inhibition of tumor growth and ultimately improved overall survival in mice harboring HER2/neu amplified carcinosarcoma xenografts.

A large volume of data is available regarding the targeting of HER2/neu in a number of cancers using the antibody trastuzumab. Trastuzumab acts at the level of the extracellular domain of the HER2/neu receptor through two main mechanisms. Once bound to the receptor it causes antibody dependent cytotoxicity and to a lesser extent decreased receptor dimerization and signaling through the MAPK and PI3K/AKT pathways [16]. Unlike trastuzumab, small tyrosine kinase inhibitors, including neratinib, exploit the ATP binding pocket of the tyrosine kinases within the cell, allowing for more robust signal inhibition. They possess a unique molecular structure that allows them specificity to covalently bind within these pockets. Neratinib specifically targets a conserved cytosine residue in both ErbB1 and HER2, and covalently binds to this site through a Michael reaction allowing for

less cytotoxic side effects than might be expected when using an irreversible inhibitor [17]. Previously published preclinical data in HER2/neu amplified uterine serous carcinoma suggest that neratinib may be a highly efficacious treatment modality in patients harboring chemotherapy resistant gynecologic malignancies that harbor HER2/neu amplification [10]. This study provides the first preclinical data regarding Neratinib's potential efficacy in the treatment of HER2/neu amplified uterine and ovarian carcinosarcomas.

Neratinib taken as a once daily tablet has been evaluated in multiple phase 1 trials. Studies revealed that the most common side effects included diarrhea and fatigue with dose limiting toxicities including diarrhea and anorexia [18]. Neratinib has also been found to be a safe and efficacious treatment in HER2 amplified breast cancers when given in combination with cytotoxic agents including paclitaxel and vinorelbine. [19], [20]. Given the overall tolerability of the drug, its specificity, and potent signaling inhibition properties, neratinib was evaluated further in clinical trials to determine its potential efficacy.

In a recent phase II study neratinib provided an objective response rate of 24% in patients with HER2 amplified breast cancers that were previously treated with trastuzumab, while trastuzumab naïve patients treated with neratinib had an objective response rate of 56% [21]. These data suggest that neratinib is efficacious in the treatment of metastatic HER2 amplified breast cancer and is able to generate clinical responses in patients who previously had progression of disease while being treated with trastuzumab. More recently data from the phase 2 ISPY 2 trial were presented at the 2014 American Association for Cancer Research, which described improved complete pathologic response in patients with HER2 positive breast cancers that received neoadjuvant neratinib in combination with paclitaxel compared to trastuzumab with paclitaxel, 55% vs. 32% respectively [22]. These data again suggest that the unique mechanism of action of neratinib allows for greater response rates in patients with HER2 amplified breast cancer. Given the results of these studies and others a number of phase III trials are planned or underway in colon (NCT0196002), breast (NCT01808573, NCT01111825, NCT01008150, NCT01042379, NCT01494662, NCT01670877) and non-small cell lung cancer (NCT01827267). The preclinical data presented here suggest for the first time that neratinib may be an efficacious treatment for HER2 amplified carcinosarcoma. These data combined with knowledge gained from clinical trials in breast cancer, make neratinib an attractive potential treatment for HER2 amplified carcinosarcoma, which should be explored in clinical trials.

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HIGHLIGHTS

- This is the first research article to evaluate the efficacy of neratinib in ovarian and uterine carcinosarcomas.
- Neratinib may represent a novel therapeutic agent for ovarian and uterine carcinosarcomas.
- HER2/neu amplification determine sensitivity of ovarian and uterine carcinosarcomas to neratinib

A

	SARARK6*	SARARK4	SARARK7	SARARK9*	SARARK1	SARARK8	SARARK10
Mean	0.01987	0.07897	0.2036	0.00478	0.2387	0.09659	0.2189

Figure 1A.

Comparison of the IC₅₀ values, in response to neratinib, of HER2/neu amplified cell lines SARARK6 and SARARK9 to HER2/neu non-amplified cell lines *in vitro*.

Figure 1B.

Comparison of the IC₅₀ values of HER2/neu amplified cell lines SARARK6 and SARARK9 sensitivity to neratinib in vitro compared to HER2 non amplified cell lines.

A **Figure 2A.**

Effect of 0.065 μ M neratinib on HER2/neu autophosphorylation over time in HER2/neu amplified SARARK6.

Figure 2B.
Effect of 0.065 μ M neratinib on phosphorylation of transcription factor S6 over time in HER2/neu amplified SARARK6.

Figure 2C.

Effects of both very low and physiologically attainable concentrations of neratinib on HER2/neu autophosphorylation at 8 hours in HER2/neu amplified SARARK6.

Figure 2D.

Effects of both very low and physiologically attainable concentrations of neratinib on downstream phosphorylation of the transcription factor S6 at 12 hours in HER2/neu amplified SARARK6.

Figure 3.

Neratinib causes arrest of the cell cycle in G0/G1 with a significant effect seen with both 0.065 μ M and 0.133 μ M of drug.

A**SARARK6 (HER2/Neu 3+) *In vivo* tumor volume** **Figure 4A.**

Neratinib dramatically inhibits tumor growth in vivo with significant effect seen as early as 9 days after treatment began ($p=0.012$) with the greatest difference noted at 30 days ($p<0.0001$).

Figure 4B.

Treatment with neratinib also significantly improved overall survival compared to vehicle treated mice, $p=0.0039$.

Table 1

Characteristics and demographic data of the 7 primary carcinosarcoma cell lines used.

Cell Line	Age	Race	FIGO Stage	Primary Site	IHC Cell Block	FISH	Histology
SARARK6	78	C	IV/IIB	Ovary	3+	Amplified	Homologous
SARARK4	77	C	IIIC	Ovary	0	Non Amplified	Heterologous
SARARK7	55	AA	IV	Ovary	1+	Non Amplified	Heterologous
SARARK9	66	C	IIIC	Uterus	3+	Amplified	Homologous
SARARK1	70	AA	IC	Uterus	0	Non Amplified	Homologous
SARARK8	46	C	IIB	Uterus	0	Non Amplified	Homologous
SARARK10	62	C	IVB	Uterus	1+	Non Amplified	Homologous

Legend: AA=African American, C= Caucasian, FIGO= International Federation of Gynecology and Obstetrics, IHC= Immunohistochemistry, FISH= Fluorescent *in situ* hybridization.