Nestin: A Biomarker of Aggressive Uterine Cancers

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Cancers of the uterine corpus are the most common gynecologic cancers in the United States with incidence and mortality increasing both in the United States of America and globally [1,2]. Most uterine cancers have a favorable prognosis and are cured by surgery alone. Identifying patients at risk of recurrence, particularly those misclassified as low risk by current clinicopathologic standards [3-9] is of paramount importance. Physicians need better risk stratification tools to identify when and which adjuvant therapy will decrease recurrence. Validated biomarkers with prognostic, predictive, and/or theragnostic value will likely play a vital role in advancing assessments of risk and guiding clinical management decision making.

Objective Evidence of potential prognostic and predictive value for nestin was investigated in well-annotated uterine cancers (UCs). Methods Nestin expression and previously-published biomarkers were evaluated by immunohistochemistry (IHC) in UC tissue microarrays. Biomarkers were categorized as low vs. high, and nestin was cut at 10% positive staining. Relationship between nestin and clinicopathologic factors, biomarkers and outcome were evaluated using exact/log-rank testing or logistic/Cox modeling. Results There were 323 eligible cases, 34% had advanced stage disease, 37% had type II disease, and 5% were carcinosarcomas. High nestin, observed in 19% of cases, was more common in advanced vs. early stage disease, type II cancers or uterine carcinosarcoma vs. type I cancers, grade 3 disease, positive lymphovascular space invasion (LVSI) and tumors > 6 cm (p < 0.05). Nestin was inversely correlated with ER, PR and TFF3, and correlated with p53 and IMP3. Women with high vs. low nestin had worse progression-free survival (PFS) and cancer-specific survival overall, and worse PFS in the subset who received no adjuvant therapy or radiation, or had early stage, type I disease or tumors with both low and high ER, PR, TFF3, PTEN, p53 or IMP3. The relationship between nestin and PFS was independent of stage, LVSI and risk categorization but not type of UC. Conclusions High nestin was more common in UCs with aggressive features and poor outcome. Nestin may

represent a predictive biomarker for treatment selection for patients previously considered to be lower risk and a candidate for no or radiation-based adjuvant therapy, and compliment ER/PR testing.

Nestin is a class VI intermediate filament protein that was first described as a neural stem marker [10] and regulates the TGFβ (transforming growth factor beta) pathway [11]. It plays a critical role in cancer cell migration, invasion, angiogenesis, and metastasis both in vitro and in vivo [12-15]. Nestin confers a poor prognosis in a number of cancers [11,16-18], but had not yet been explored in uterine cancer.

The Gynecologic Cancer Center of Excellence (GYN-COE) consortium and members of the John P Murtha Cancer Center (MCC) worked with a Leading Gynecologic Pathologist (PMF) to evaluate immunohistochemical expression levels of nestin with previouslypublished biomarkers of endometrial cancer in a well-annotated cohort of 323 uterine cancer patients. Prognostic, predictive, and theragnostic utility were assessed using classic biostatistical methods and the existing diagnostic, patient outcomes, cancer treatment, and biomarker data for this cohort. Patients were classified into lower versus (vs.) higher risk groups based on the available surgicopathologic characteristics. Normalized level 3 RNA next generation sequencing (RSEM) expression data for nestin and the endometrial cancer biomarkers were also extracted along with clinical and outcome data from The Cancer Genome Atlas (TCGA) for patients with uterine corpus endometrial carcinoma.

High Immunohistochemical (IHC) expression of nestin, defined as >10% positive tumor cells staining, was observed in 19% of uterine cancer cases and was associated with aggressive disease characterized by advanced stage, non-endometrioid cell type including carcinosarcomas, high grade, tumor size 6+cm, and presence of Lymphovascular Space Invasion (LVSI). Advanced stage cancers and those with LVSI were twice as likely to exhibit high tumor staining for nestin. Patients with higher risk disease (grade 3 disease or

nonendometrioid cell type) were 5 times more likely to exhibit high IHC tumor nestin expression [19].

Nestin expression was inversely associated with Estrogen Receptor (ER) and Progesterone Receptor (PR) expression, which are more common in low risk, type I endometrial cancer. In contrast, nestin expression was directly associated with tumor protein 53 (p53) which is more common in high risk, type II endometrial cancer [19].

Nestin expression was associated with worse Progression-Free (PFS), Cancer-Specific (CSS), and Overall Survival (OS). The relationship between nestin and clinical outcomes was independent of stage, lymphovascular space invasion (LVSI) or risk categorization [19].

High nestin expression also predicted risk of disease progression within 5 years with an accuracy of 60%. The accuracy of predicting risk of disease progression within 5 years increased to 0.8 by combining nestin with tumor stage and type of uterine cancer. The addition of other biomarkers including ER, PR, and p53 to nestin, stage, and type of uterine cancer did not increase predictive accuracy further and in fact accuracy dropped below 63% [19].

A positive interaction test demonstrated that IHC staining for nestin had potential theragnostic value in uterine cancer patients who receive no adjuvant therapy or radiation therapy. High vs. low IHC expression of nestin indicated worse PFS in the subset of patients who either did not receive adjuvant therapy or who had adjuvant radiation only, but not in the patient treated with chemotherapy or chemoradiation [19].

Data from TCGA uterine cancer project was assessed to evaluate whether transcript expression of nestin maintained prognostic significance, however nestin did not exhibit prognostic value at the RNA-seq level. High IHC staining of p53 was associated with worse PFS, though higher transcript expression of TP53 (p53) was associated with improved PFS and a reduced risk of disease progression in uterine cancers and the subset of endometrioid. Consistent with IHC-based results, ERS1 (ER) and PGR (PR) were associated with better PFS and a reduced risk of disease progression in uterine cancers. Nestin did not retain prognostic value at the transcript level, however there are numerous post-transcriptional modifications that may occur to explain this disparity. In addition, TP53 did not exhibit its known poor prognostic effect at the RNASeq level. Tumor purity may also be a confounding factor on transcript expression and disparities with IHC staining that has been demonstrated across a multitude of samples in many different TCGA cancer types [20].

High nestin expression was more common in tumors with aggressive features and women with disease progression or cancer death. Nestin was inversely correlated with the type I biomarkers, ER and PR, and directly associated with the type II biomarker, p53. High vs. low IHC expression of nestin was a poor prognostic factor for worse PFS, CSS, and OS. Nestin retained independent prognostic value for clinical outcomes when controlling for stage, LVSI or risk categorization.

The predictive accuracy of nestin for disease progression was 60% alone and increased to 80% when combined with stage and uterine cancer type. A predictive accuracy of 80% is very promising given that Bendifallah et al. recently illustrated that PORTEC-1, GOG-99, SEPAL, ESMO, and ESMO-modified had a predictive accuracy for recurrence of 0.68, 0.65, 0.66, 0.71 and 0.73, respectively [9]. Our clinical combination with nestin merits further research and investigation to validate and enhance the performance of this predictor.

Uterine cancer is an increasing public health concern and biomarkers are needed to enhance the personalized care of patients. Nestin lends prognostic and predictive value and may be useful as a theragnostic biomarker for endometrial cancer patients with low or intermediate risk who receive no adjuvant therapy or radiation therapy. Improvements in risk stratification are clearly needed to better guide clinical management and care. This may be particularly important in patients, currently classified as low or intermediate risk, who will receive either no adjuvant therapy or radiation alone as nestin portended worse prognosis in this subset. These patients may benefit from more aggressive adjuvant treatment to prevent recurrence. Because most uterine cancer patients are considered low risk, predictive assessment using nestin with clinical factors has the potential to benefit a significant number of patients worldwide.

In this study, nestin was associated with aggressive tumor characteristics and worse clinical outcome, which is consistent with previous mechanistic studies of nestin in other cancers. This biomarker, monitoring using an IHC

assay, holds promise as a prognostic and predictive factor that may also have theragnostic value as a companion triage tool to identify lower risk patients who may require more aggressive clinical treatment.