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The Prognostic Value of Tumor-Associated Macrophages in Leiomyosarcoma:

A Single Institution Study

Kristen N. Ganjoo, MD[*,†], **Daniela Witten, MS**[‡], **Manisha Patel**[*], **Inigo Espinosa**[§], **Trang La, MD**[¶], **Rob Tibshirani, PhD**[‡], **Matt van de Rijn, MD**[§], **Charlotte Jacobs, MD**[*], and **Robert B. West**[**,§]

[*]Division of Oncology, Department of Medicine, Stanford University Medical Center, Stanford, CA

[†]Department of Medicine, Palo Alto Veterans Affairs Health Care System, Palo Alto, CA

[‡]Department of Statistics, Stanford University, Stanford, CA

[§]Department of Pathology, Stanford University Medical Center, Stanford, CA

[¶]Department of Radiation Oncology, Stanford University, Stanford, CA

[‖]Department of Health Research and Policy, Stanford University, Stanford, CA

[**]Department of Pathology and Laboratory Service, Palo Alto Veterans Affairs Health Care System, Palo Alto, CA

Abstract

Introduction—High numbers of tumor-associated macrophages (TAMs) have been associated with poor outcome in several solid tumors. In 2 previous studies, we showed that colony stimulating factor-1 (CSF1) is secreted by leiomyosarcoma (LMS) and that the increase in macrophages and CSF1 associated proteins are markers for poor prognosis in both gynecologic and nongynecologic LMS in a multicentered study. The purpose of this study is to evaluate the outcome of patients with LMS from a single institution according to the number of TAMs evaluated through 3 CSF1 associated proteins.

Methods—Patients with LMS treated at Stanford University with adequate archived tissue and clinical data were eligible for this retrospective study. Data from chart reviews included tumor site, size, grade, stage, treatment, and disease status at the time of last follow-up. The 3 CSF1 associated proteins (CD163, CD16, and cathepsin L) were evaluated by immunohistochemistry on tissue microarrays. Kaplan-Meier survival curves and univariate Cox proportional hazards models were fit to assess the association of clinical predictors as well as CSF1 associated proteins with overall survival.

Results—A total of 52 patients diagnosed from 1983 to 2007 were evaluated. Univariate Cox proportional hazards models were fit to assess the significance of grade, size, stage, and the 3 CSF1 associated proteins in predicting OS. Grade, size, and stage were not significantly associated with survival in the full patient cohort, but grade and stage were significant predictors of survival

Reprints: Kristen N. Ganjoo, MD, Department of Medicine, 875 Blake Wilbur Drive, Stanford, CA 94305. kganjoo@stanford.edu..

in the gynecologic (GYN) LMS samples ($P = 0.038$ and $P = 0.0164$, respectively). Increased cathepsin L was associated with a worse outcome in GYN LMS ($P = 0.049$). Similar findings were seen with CD16 ($P < 0.0001$). In addition, CSF1 response enriched (all 3 stains positive) GYN LMS had a poor overall survival when compared with CSF1 response poor tumors ($P = 0.001$). These results were not seen in non-GYN LMS.

Conclusions—Our data form an independent confirmation of the prognostic significance of TAMs and the CSF1 associated proteins in LMS. More aggressive or targeted therapies could be considered in the subset of LMS patients that highly express these markers.

Keywords

tumor-associated macrophages; leiomyosarcoma

Current pathologic grading systems do not reliably predict outcome in patients with leiomyosarcoma (LMS). Tumors from the same sites that appear similar by histology may vary greatly in response to therapy and survival. The presence of tumor-associated macrophages (TAMs) in some carcinomas and lymphomas is associated with poor prognosis.[1,2]

There is evidence that LMS cells secrete cytokines that attract and stimulate TAMs. Colony stimulating factor-1 (CSF1) is a cytokine that induces the proliferation and differentiation of macrophages and monocytes.[3–5] Our group has recently described a CSF1 response signature that involves a number of genes including 3 proteins involved with TAMs.[6,7] These include: CD16 (FcγRIIIa), CD163 (scavenger receptor cystein-rich [SRCR] member), and cathespin L (CTSL).

In 2 different studies, we examined the presence of macrophages and the macrophage response. In one study, we found that increased numbers of macrophages is associated with a worse outcome in nongynecologic (non-GYN) LMS but not in GYN LMS.[8] In a second study, we showed that in some LMS tumors, CSF1 is secreted by the tumor cells and that the expression of CSF1 and the coordinated expression of 3 associated proteins predict outcome in both GYN and non-GYN LMS.[6] These studies were done on the same set of cases using a tissue microarray with 149 cases of LMS collected from multiple institutions across the United States with different treatment algorithms.

Here we report a single institution retrospective study on an independent set of 52 patients treated at Stanford University Medical Center to evaluate the effect of TAMs on the outcome of patients with LMS utilizing the 3 CSF1 associated proteins.

PATIENTS AND METHODS

Patients with LMS treated at Stanford University with archived tissue and clinical data were eligible for this retrospective study. Data from chart review included tumor site, location of primary (GYN vs. non-GYN), size, grade, stage, treatment, and disease status at the time of last follow-up.

A tissue microarray was generated with material from all 52 patients. Protein expression of the 3 CSF1 response genes (CD163, CD16, and CTSL) was assessed by immunohistochemistry (IHC) on the LMS tissue microarray. These studies were interpreted by 2 pathologists (R.W., I.E.) using scoring criteria specifically defined for each marker.[6] The number of macrophages was recorded in 1 of 4 bins, based on the number of cells/high power field (HPF): (1) up to 10 cells, (2) more than 10 cells and up to 20 cells, (3) more than 20 cells and up to 45 cells, (4) more than 45 cells. We discretized the protein expression levels into "positive" and "negative," as follows: for CD16 and CTSL, positive was defined as those with more than 10 cells/HPF, whereas for CD163, positive was defined as those with more than 45 cells/HPF. The results of the protein expression from these 3 genes were used to divide the LMS cases into "CSF1 response enriched" (all 3 proteins positive) and "CSF1 response poor" groups.

Statistics

Univariate Cox proportional hazards models were fit to predict overall survival (OS) using number of predictors: stage, grade, and size of tumor, as well as CTSL, CD163, and CD16 expression (treating the binned number of cells described in the previous section as a continuous variable). Each model was fit on 3 sets of patients: the full patient set, the subset of patients with GYN LMS, and the non-GYN LMS patients. In addition, for each protein and for each set of patients, Kaplan-Meier survival curves were made by discretizing the expression of each protein, as described in the previous section. Finally, patients with all 3 proteins positive were compared with the rest of the patients, using a Cox proportional hazards model and Kaplan-Meier survival curves.

RESULTS

A total of 52 patients diagnosed and treated at Stanford University from 1983 to 2007 were included in this analysis. Patient characteristics are listed in Table 1 along with the predictors used in each of the statistical analyses performed.

Univariate Cox proportional hazards models were fit using size, grade, and stage as predictors. For each Cox model, the coefficient beta, the *P*-value, and the associated sample size are reported in Table 2. Score statistics for grade, size, and stage were not significant on the full set of patients; however, grade and stage were significantly associated with survival in the GYN LMS patients (Table 2).

There was a nonsignificant trend toward superior survival in patients with CD163 (evaluated by IHC, Fig. 1A) TAM <45 (40%, 5-year) compared with those with TAM ≥45 (28%, 5-year). Similarly, score statistics for the Cox proportional hazards models using CD163 as a predictor were not significant (Table 2).

CD16 (Fig. 1B) and CTSL (Fig. 1C) were evaluated by IHC. Increased levels of CD16 were associated with decreased OS in GYN LMS ($P < 0.0001$) (Fig. 2A). This was not seen in non-GYN LMS (Fig. 2B). Increased CTSL in GYN LMS was also associated with a worse outcome ($P = 0.049$) (Fig. 3A). This was not the case in non-GYN LMS (Fig. 3B).

Finally, OS was evaluated in tumors with all 3 CSF1 associated proteins present in the sample. As mentioned earlier, CSF1 enriched samples were defined as having ≥10 CTSL, ≥10 CD16, and ≥45 CD163. CSF1 enriched GYN LMS tumors had a worse outcome compared with CSF1 response poor groups ($P = 0.001$) (Fig. 4A). This effect on outcome was not seen in non-GYN LMS (Fig. 4B).

DISCUSSION

The current grading system for LMS does not adequately define the prognosis in these tumors. Although grade, site, and size affect prognosis, patients with similar tumor characteristics often have different survival rates. We sought other pathologic prognosticators to aid in therapy decisions for LMS patients. We have categorized LMS into 2 groups depending on the organ of origin (GYN or non-GYN). In a previous study, we found that the presence of TAMs in the tumor microenvironment is associated with poor prognosis in non-GYN LMS.[8] In that study, CD163- and CD68-positive macrophages were significantly associated with worse survival in non-GYN LMS. In a subsequent study, we showed that CSF1 is expressed by LMS tumor cells and that the expression of CSF1 and the 3 associated proteins predicted worse outcome in both GYN and non-GYN LMS.[6] These studies were performed on the same group of 149 LMS cases collected from institutions across the United States.

In the current study, we evaluated the role of TAMs in LMS using the expression of the 3 associated proteins on cases seen at a single institution, to address their utility as a prognostic marker in a typical clinical setting. No reliable antibodies exist for CSF1 and in prior studies this marker was detected by in situ hybridization, a technique not generally available in clinical laboratories. We therefore decided to analyze the set of LMS patients using only the 3 CSF1 associated markers (CD163, CD16, and CTSL) that can be detected by routine IHC. Analysis of a set of cases from a single institution, with a standard way of diagnosis, work up, treatment, and outcome reporting also generates results more consistent with Stanford University's routine clinical practice. Moreover, with a more homogenous set of cases, we could evaluate the prognostic strength of individual markers to begin to address which markers would be part of a clinically robust assay.

In our study, we evaluated the univariate association of each of the 3 CSF1 signature associated proteins with survival. Increased levels of CD163 were somewhat associated with decreased survival, though the association was not statistically significant. High numbers of CD16 and CTSL positive TAMs were significantly associated with decreased survival in GYN LMS. We did not find a significant correlation between high levels of the proteins and outcome in non-GYN LMS. Our initial study on the presence of macrophages in LMS found significantly worse outcome in only non-GYN LMS, whereas there was a trend for a correlation with worse outcome in GYN LMS. In the subsequent study where we examined the expression of CSF1 and the 3 associated proteins, we found that the co-ordinated expression of these 4 markers was associated with a worse outcome in both GYN and non-GYN LMS. Despite the differences between the prior 2 studies and the current one, we hypothesize that these findings on the 52 Stanford patients are essentially consistent with our 2 previous studies. Our current single institution has fewer patients, which analyze the same

set of 149 cases, and this may be the reason that no survival changes are seen for the non-GYN cases. Moreover, though not statistically significant, the study reported by Lee et al did find a trend toward worse outcome in GYN cases.[8]

We and others have shown that the CSF1 macrophage response affects prognosis in cancers other than LMS. In breast cancer, we have found that the CSF1 macrophage response as measured by gene expression profiling is associated with outcome in a complex manner involving estrogen receptor status.[7] Another study found that expression of just CTSL is valuable in determining disease-free survival and response to adjuvant therapy in patients with breast cancer.[9] In prostate cancer, researchers have found that the CSF1 macrophage response also has an influence on outcome.[10] In that study, CSF1, CSF1R, and CD68 macrophages were evaluated in metastatic versus localized prostate cancers. The CSF1 and CSF1R expression was higher in metastatic prostate cancer suggesting a role in tumor progression. In addition, the number of CD68-positive macrophages was higher in the metastasis versus the primary tumors; however, there were higher numbers in localized prostate cancer compared with metastatic cancer.

CSF1 is a cytokine that can be produced by certain tumors and can attract macrophages through binding to their CSF1 receptor. This receptor is expressed not only on macrophages but can also be found on tumor cells. Our group has recently discovered a CSF1 response signature that involves a number of genes including: CD16 (FcγRIIIa), CD163 (SRCR member), and CTSL.[7] CD16 (FcγRIIIa) is a receptor for the Fc of IgG which has been described in macrophages, natural killer cells, and neutrophils. CD16 plays a key role in antibody dependent cellular cytotoxicity-mediated cancer cell death by natural killer cells.[11] CD163 is a member of the SRCR superfamily expressed on most mature macrophages which functions in binding of hemoglobin-haptoglobin complexes. Furthermore, it plays an important role in the resolution of tissue inflammation.[12] CTSL, a lysosomal cystein protease highly expressed in macrophages, is responsible for the degradation and turnover of intracellular proteins.[13]

The biology of the role of TAMs in the progression and metastasis of tumors has been extensively studied in solid tumors. TAMs are attracted to the tumor microenvironment by tumor secreted CSF1. TAMs in turn secrete epidermal growth factor (EGF). EGF is a chemotactic factor which promotes invasion through binding to epidermal growth factor receptor (EGFR) on the tumor cells (paracrine loop).[14] Goswami et al reported that tumor invasion in vivo can be interrupted by either blocking CSF1R on TAMs or EGFR on tumor cells. Blocking the CSF1R on the TAMs can interrupt the autocrine loop by preventing CSF1 binding and blocking tumor growth.

In our study, the presence of TAMs in GYN LMS had a negative impact on survival. This finding suggests a novel treatment approach for these tumors as TAMs have become a potential target in sarcoma therapy. For example, trabectedin (Yondelis) has shown activity in sarcomas by inhibiting TAMs differentiation and inflammatory cytokine production.[15] Another strategy to block the activity of TAMs is by blocking the EGF secreted by TAMs to binding to EGFR on the tumor cells (erlotinib) which has not yet been adequately studied in LMS.

Our study represents an independent cohort confirmation of the prognostic value of the CSF1 macrophage association in LMS. These findings provide additional support for the use of a CSF1 macrophage assay in the clinical setting to identify patients with poor prognosis LMS. At the time of diagnosis, patients with CSF1 enriched GYN LMS could be considered for more aggressive treatments with intensive chemotherapy or even with new therapies targeted at the CSF1 macrophage interaction.

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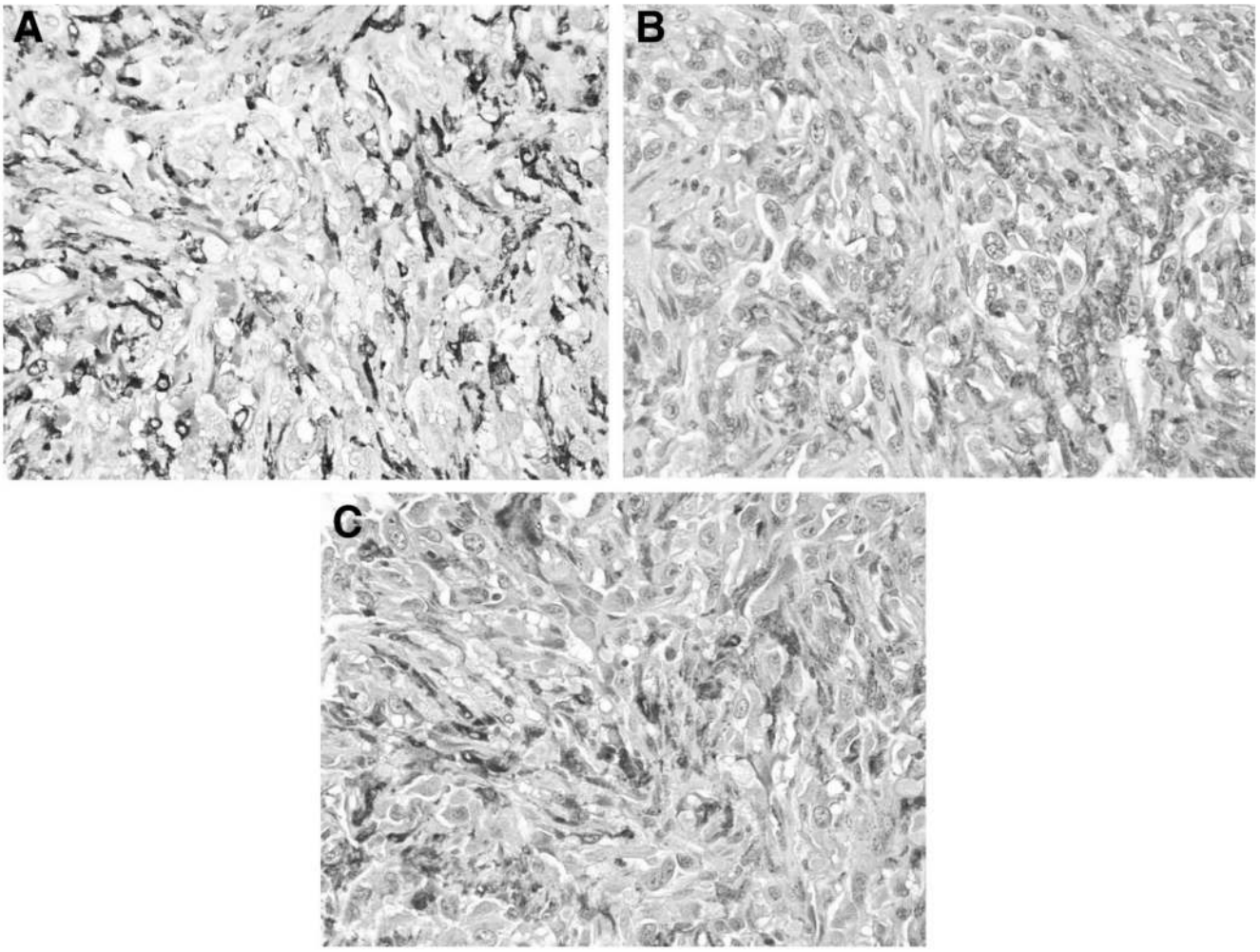

FIGURE 1.
A, CD163; (B) CD16; (C) cathepsin L (CTSL) immunostains (×40 per high power field).

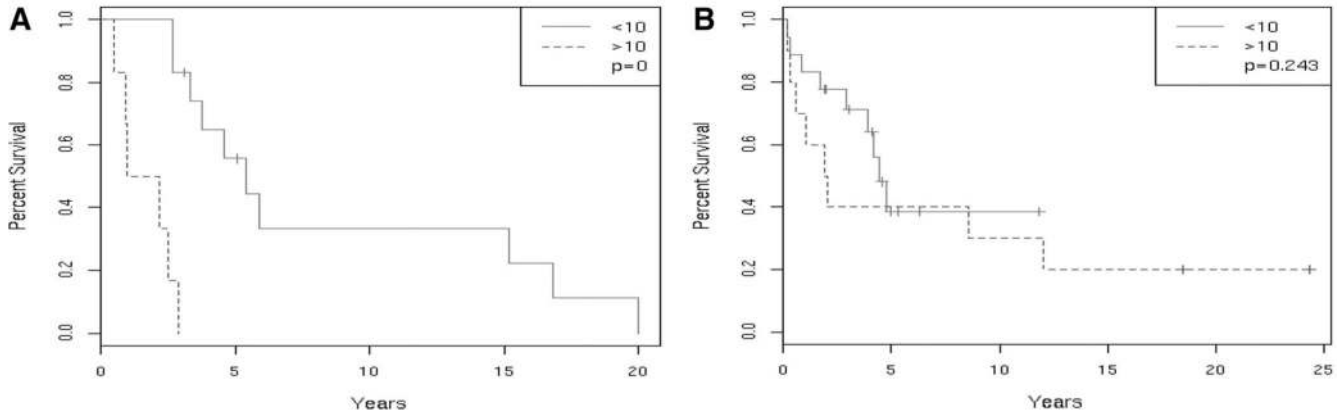

FIGURE 2.

CD16 and survival in (A) gynecologic (GYN) leiomyosarcoma (LMS), (B) non-GYN. Kaplan-Meier survival curves are shown for the positive versus negative comparison for each protein. The reported *P*-values are for the score statistic for the Cox proportional hazards model, using the predictors as defined in Table 1. A, CD16 in GYN LMS (n = 18). B, CD16 in non-GYN LMS (n = 28).

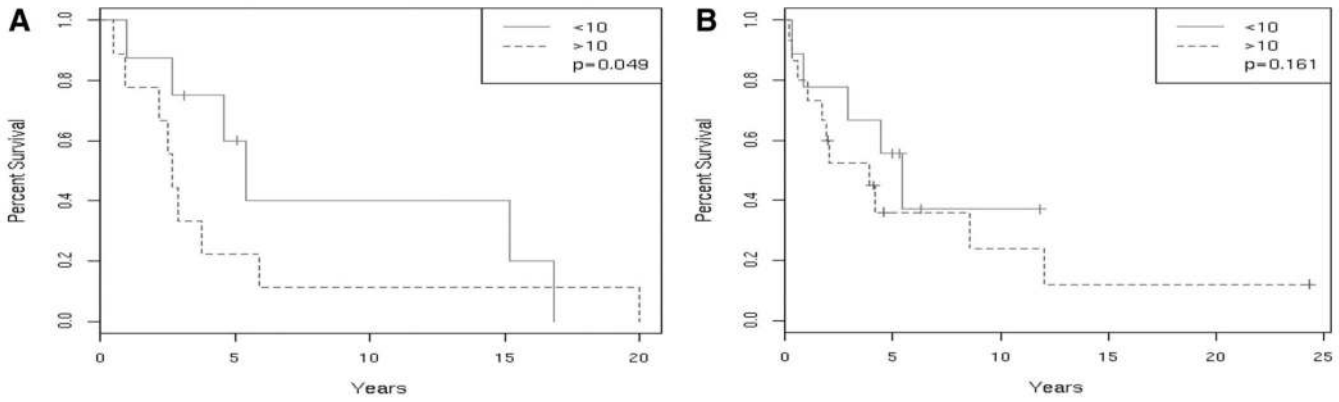

FIGURE 3.

CTSL and survival in (A) GYN LMS, (B) non-GYN. Kaplan-Meier survival curves are shown for the positive versus negative comparison for each protein. The reported *P*-values are for the score statistic for the Cox proportional hazards model, using the predictors as defined in Table 1. A, CTSL in GYN LMS (n = 17). B, CTSL in non-GYN LMS (n = 24).

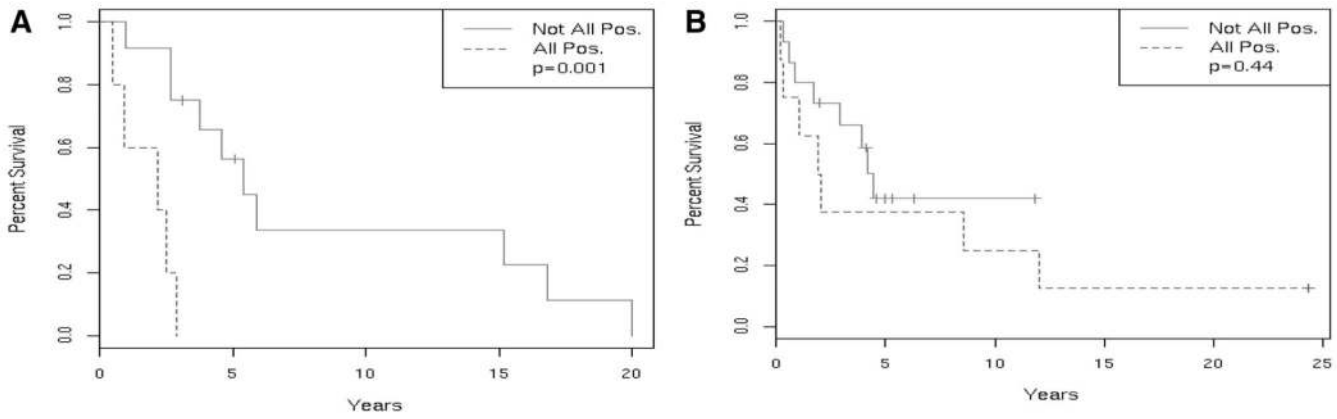

FIGURE 4.

CD163, CD16, CTSL positive versus negative with survival correlation (A) GYN; (B) non-GYN. Kaplan-Meier survival curves are shown for the positive versus negative comparison for each protein. The reported *P*-values are for the score statistic for the Cox proportional hazards model, using the predictors as defined in Table 1. A, CD163, CD16, CTSL in GYN LMS (n = 17). B, CD163, CD16, CTSL in non-GYN LMS (n = 23).

TABLE 1

Patient Characteristics (n = 53)

	Number (%)	Value of Predictor in Cox Model
Age		
Median	54 yr	
Range	(24–90 yr)	
Gender		
Male	13 (25%)	
Female	40 (75%)	
Race		
White	42 (79%)	
African American	5 (9%)	
Asian	2 (4%)	
Other	4 (8%)	
Tumor size		
<5 cm	9 (17%)	1
5–10 cm	16 (30%)	2
>10 cm	24 (45%)	3
Unknown	4 (8%)	
Tumor grade		
Low	11 (21%)	1
High	41 (77%)	2
Unknown	1 (2%)	
Stage		
I	5 (10%)	1
II	6 (11%)	2
III	26 (49%)	3
IV	15 (28%)	4
Unknown	1 (2%)	
Location		
GYN	19 (36%)	
Non-GYN	34 (64%)	
CTSL		
<10 cells	17 (32%)	1
≥10 cells	13 (25%)	2
≥20 cells	2 (4%)	3
≥45 cells	9 (17%)	4
Unknown	12 (23%)	
CD163		
<10 cells	3 (6%)	1
≥10 cells	6 (11%)	2
≥20 cells	9 (17%)	3

	Number (%)	Value of Predictor in Cox Model
≥45 cells	27 (51%)	4
Unknown	8 (15%)	
CD16		
<10 cells	30 (56%)	1
≥10 cells	0 (0%)	2
≥20 cells	7 (13%)	3
≥45 cells	9 (17%)	4
Unknown	7 (13%)	
CSF1 response enriched *		
Not all proteins positive	27 (51%)	1
All proteins positive	13 (25%)	2
Unknown	13 (25%)	

* All proteins positive is defined as CD163 ≥45, CTSL ≥10, and CD16 ≥10.

TABLE 2

Univariate Cox Proportional Hazards Models

Predictor	All Samples	GYN Only	Non-GYN Only
Grade	[β] = 0.612, P = 0.204, n = 52	[β] = 1.47, P = 0.04, n = 19	[β] = −0.17, P = 0.82, n = 33
Size	[β] = 0.256, P = 0.28, n = 49	[β] = 0.334, P = 0.32, n = 18	[β] = 0.284, P = 0.42, n = 31
Stage	[β] = 0.359, P = 0.101, n = 52	[β] = 0.80, P = 0.016, n = 19	[β] = 0.016, P = 0.96, n = 33
CTSL	[β] = 0.343, P = 0.023, n = 41	[β] = 0.51, P = 0.049, n = 17	[β] = 0.284, P = 0.16, n = 24
CD16	[β] = 0.322, P = 0.02, n = 46	[β] = 0.88, P < 0.001, n = 18	[β] = 0.235, P = 0.24, n = 28
CD163	[β] = 0.1, P = 0.64, n = 45	[β] = −0.186, P = 0.55, n = 18	[β] = 0.132, P = 0.26, n = 27
CSF1 enriched	[β] = 0.647, P = 0.084, n = 40	[β] = 2.2, P < 0.001, n = 17	[β] = 1.53, P = 0.44, n = 23