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Prognosis of macrophage density in the absence of neutrophils in differentiated thyroid cancer

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Abstract

Background: Despite the advances in treatment of differentiated thyroid cancer (DTC), predicting prognosis remains a challenge. Immune cells in the tumor microenvironment may provide an insight to predicting recurrence. Therefore, the objective of this study was to investigate the association of tumor-associated macrophages (TAMs) and tumor-associated neutrophils (TANs) with recurrence in DTC and to identify serum cytokines that correlate with the presence of these immune cells in the tumor.

Materials and Methods: Forty-two DTC tissues from our institutional neoplasia repository were stained for immunohistochemistry markers for TAMs and TANs. Additionally, cytokine levels were analyzed from these patients from preoperative blood samples. TAM and TAN staining were compared to clinical data and serum cytokine levels.

Results: Neither TAM nor TAN scores alone correlated with tumor size, presence of lymph node metastases, multifocal tumors, lymphovascular or capsular invasion, or presence of BRAFV600E mutation (all $p>0.05$). There was no association with recurrence-free survival (RFS) in TAN density (mean RFS 169.1 vs 148.1 months, $p=0.23$) or TAM density alone (mean RFS 121.3 vs 205.2 months, $p= 0.54$). However, when scoring from both markers were combined, patients with high TAM density and TAN negative scores had significantly lower RFS (mean RFS 50.7 vs 187.3

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Author Contribution: This study was designed and conceptualized by AEO, LAS, JEP, AT. Data were acquired by LS, AEO, LAS and CE. Analysis and interpretation of data were performed by CS, AEO, LAS, AT. The manuscript was initially drafted by AEO, LAS, with critical revision by JEP, CS, AT for important intellectual content. The final draft was approved by AEO, LAS, CS, CE, LS, JEP, AT

Declaration of Interest

The authors declare no conflict of interest. Additionally, the authors maintain full control of all primary data included in this article and will make it available for review if requested.

months, p=0.04) compared to the remaining cohort. Patients with high TAM/negative TAN tumors had significantly lower serum levels of IL-12p70, IL8, TNF-alpha and TNF-beta.

Conclusion: In differentiated thyroid cancers, high density of TAMs in the absence of TANs is associated with worse outcome. Assessment of multiple immune cell types and serum cytokines may predict outcomes in DTC.

Keywords

tumor associated macrophages; tumor associated neutrophils; thyroid cancer; cytokines

Introduction

Thyroid cancer is the most common endocrine cancer, with an increasing incidence in the past decade, now accounting for approximately 3.8% of all new cancer cases in the United States (1). By 2030, thyroid cancer is projected to be the 2nd most common cancer in women and 4th most common cancer overall (2). Some investigators have suggested that the introduction and the subsequent widespread use of ultrasonography and fine-needle aspiration biopsy, along with the increased use of diagnostic imaging modalities, such as CT, MRI and PET, has led to increased detection of small thyroid nodules and diagnosis of thyroid cancer at an early stage (3-5). Despite favorable long-term survival in differentiated thyroid cancers, recurrence remains a major concern with 40-yr recurrence rates reported as high as 35%, two thirds of which occurred within the first decade after initial therapy (6). Some of the prognostic factors currently used in predicting recurrence include age at diagnosis, size of primary tumor, presence of soft tissue invasion or distant metastasis, American Thyroid Association (ATA) risk stratification guideline as well other information such as mutational analysis (7-10). With current prognostic factors and surveillance practices, attempts to predict tumor recurrence often produces inaccurate results. As such, novel markers are needed to more precisely predict disease progression. Recently, in many cancer types, attention has been directed towards the tumor microenvironment as a source for predictors of recurrence.

The tumor microenvironment (TME) is a complex milieu of cell types that communicate with one another to assist in the growth and spread of cancer. A few examples of cells found in the TME include tumor associated macrophages (TAM), tumor associated neutrophils (TAN), cancer-associated fibroblasts, pericytes and regulatory T-cells. In previous studies, increased TAM density in poorly differentiated thyroid cancer was associated with poor histologic grade, tumor invasiveness and decreased cancer-related survival (11). However, there is a paucity of data on the prognostic role of TANs, as well as TAMs in conventional DTC. As such, the objective of this study was to determine the prognostic role of both TAMs and TANs in conventional DTC. Furthermore, we sought to identify how tumor immune cell composition correlates with cytokine markers in serum from patients with DTC.

MATERIALS AND METHODS

Forty-two paraffin embedded samples of DTC were collected from the endocrine neoplasia repository of the Ohio State University Wexner Medical Center. Samples were stained via

immunohistochemistry (IHC) for macrophage marker CD163 (rabbit anti-CD163, 1:900, cat. ab87099, Abcam, Cambridge, UK) and neutrophil marker CD66b (rabbit anti-CD66b, 1:100, cat. ab197678, Abcam, Cambridge, UK) according to the following protocol. Paraffin embedded tissue was cut at 4 microns and placed on positively charged slides. Slides with specimens were then placed in a 60 °C oven for 1 hour and cooled. Slides were then placed on the Leica Bond III Autostainer. All slides are deparaffinized and rehydrated with Bond Dewax Solution (product code AR9222) and 100% alcohol. All slides were stained with in-house created protocol. Slides were quenched for 5 minutes in a 3% hydrogen peroxide solution to block for endogenous peroxidase. Antigen retrieval was performed using Leica's Bond Epitope Retrieval Solution 1 (ER1 low pH, product code AR9961) for 20 minutes. Primary antibody was incubated for 15 mins (for CD66b) or 30 mins (for CD163) at room temperature. The antibodies were detected using Leica's Bond Polymer Refine Detection system (product code DS9800). Lastly, sections were incubated with DAB mixed on-line for 10 minutes. Slides were then counterstained using Leica hematoxylin for 3 minutes then removed and dehydrated through graded ethanol solutions and coverslipped.

Expression levels were scored with the assistance of a clinical pathologist, *L.S* similarly to the method published by Fagin et al (11). Briefly, TAM density was scored numerically from 0 to 3 with 0 representing absence of macrophages per 10x high-power field (HPF) and 3 representing presence of densely populated macrophages per 10x HPF. The scoring was further collapsed into two major categories: low TAM density for samples with scores that range from 0 to 1 and high TAM density for those with scores of 2 to 3. Expression levels of TAN density was scored similarly to the methods used by Galdiero et al (12). CD66+ cells were counted and scored by a clinical pathologist blinded to patient outcomes. The median value of CD66+ counts (9 neutrophils per 10x high-powerfield) was used as a cutoff. TAN density was grouped into 2 major categories: a negative group for samples with less than 9 neutrophils per 10x HPF and a positive group for samples with greater than 9 neutrophils per 10x HPF.

Clinicopathologic data collected included gender, age at diagnosis, cancer histology, tumor size, capsular invasion, lymph node metastasis, lymphovascular invasion, multifocal tumor and BRAFV600E mutation, obtained through a database maintained within the institution's endocrine neoplasia repository. Per protocol, patient serum was collected at a preoperative office visit or on the day of surgery. A commercially available comprehensive human 30-plex cytokine panel that contained known cytokines associated with tumor immune cells including TAMs and TANs was used in this study. Patient serum was then analyzed for expression levels using Luminex ® Multiplex Assay. A waiver of consent was obtained as this was a retrospective study. The study was approved under the Ohio State University Wexner Medical Center Institutional Review Board.

Demographics and disease characteristics are reported as mean or median. Cytokine expression levels were reported as logarithm transformed values with interquartile range (IQR). Categorical variables were compared using Pearson's chi-square test. Recurrence-free survival was evaluated using Kaplan-Meier estimate, with comparisons between groups made using logrank test. Univariable and multivariable Cox regression analysis were performed to assess the effect of clinicopathologic features on recurrence. All tests of

statistical significance were twosided with level of statistical significance established at $p<0.05$. Statistical analysis was performed using SPSS v 25 (SPSS, Inc., Chicago, IL, USA).

RESULTS

Clinicopathologic characteristics

Forty-two patients were included in the cohort. Clinicopathologic characteristics are included in Table 1. The median age at diagnosis was 50 years (range 20-86). The majority of patients (80.5%) had papillary thyroid cancer as their final pathologic diagnosis while 19.5% had follicular thyroid cancer. The overall recurrence rate was 23.8% and the mean follow-up period was 61 months (IQR 11.3-92.1 months).

The univariable and multivariable Cox regression analyses of recurrence-free survival (RFS) calculated from the date of initial operation are summarized in Table 2. Age >55, and T stage IV disease were all associated with worse survival on univariable analysis while female gender was associated with improved recurrence-free survival. Multivariable analysis showed that only T stage IV disease (hazard ratio, 5.183; 95% CI, 1.225-21.924, p= 0.025) was independently associated with RFS.

Correlation of TAMs with clinicopathologic characteristics and serum cytokines

There was no significant correlation between tumor associated macrophage density and clinicopathologic factors collected (Table 3). Next, RFS based on TAM density was analyzed (Figure 1). There was no significant difference in RFS between patients with high TAM density tumors compared to low TAM density tumors (mean RFS 121.3 months vs 205.2 months, p=0.539).

Key immunoregulatory cytokines present in the patients' serum were compared to TAM tumor density (Table 4). Patients with high TAM density tumors had higher serum levels of the cytokines Mip1-alpha (p= 0.03) and IL-8 (p= 0.012). Patients with low TAM density tumors had significantly higher serum levels of MCP-1 (p=0.014).

Correlation of TANs with clinicopathologic characteristics and serum cytokines

Age >55 and female gender were significantly associated with TAN negative tumors (Table 5). There was no significant difference in RFS between patients with TAN negative tumors compared to TAN positive tumors (mean RFS 169.1 months vs 148.1 months, p=0.231, Figure 2).

Key immunoregulatory cytokines present in the patients' serum were compared to presence of TANs in the tumor (Table 6), with patients having TAN negative tumors expressing higher serum levels of IL-12p70 compared to patients with TAN positive tumors (p=0.008).

Outcome for combination of immune markers

Combined levels of these immune cells in the microenvironment were assessed because their interaction *in vivo* may play a role in the biology of the disease. Although this has not been directly shown in a tumor model, there are published data on the interaction of neutrophils

and macrophages in models of tissue inflammation (13-15). Interestingly, when scores from both markers were combined, patients with tumors that had high TAM density and were TANnegative had a significantly lower RFS compared to the rest of the cohort (mean RFS 50.7 months vs 187.3 months, p=0.04, Figure 3). We then assessed the difference in serum cytokine expression between this group and the rest of the cohort (Table 7). Patients with high TAM density/TAN negative tumors expressed lower serum levels of cytokines IL-12p70, IL-8, TNF-alpha and TNF-beta compared to the remainder of the cohort.

Discussion

Multiple studies have shown that immune cell composition of the tumor microenvironment impacts tumor development, progression and clinical outcome (11, 16-18). Tumor-associated inflammatory cells are not uncommon in thyroid cancer and have been shown to correlate with outcomes (19, 20). In our cohort, scores of TAMs or TANs alone did not correlate with aggressive histologic features or recurrence-free survival. However, when both markers were combined, we found that the presence of high TAM density in the absence of TANs is associated with worse outcomes. Furthermore, in this cohort, we found patients with high TAM density in addition to absent TAN had lower serum levels of the cytokines IL-12p70, Il-8, TNF-alpha and TNF-beta.

Previous studies have demonstrated that an increased density of TAMs is associated with tumor progression in advanced thyroid cancer (11, 21, 22). Ryder et al found that increased TAMs in poorly differentiated thyroid cancer was associated with capsular invasion, extrathyroidal extension and decreased cancer-related survival (11). In our cohort, we found that there was no significant difference in RFS between patients with high TAM density tumors compared to low TAM density tumors. Our study differs from the work of Ryder et al given that our cohort consisted of well differenced thyroid cancer. One reason why this difference potentially did not reach statistical significance in our study is secondary to relatively low sample size (n=42), as our power calculation shows that 740 patients will be needed to adequately detect a difference between the two groups.

Investigations of the clinical relevance and prognostic value of TAN density reveals differences based on cancer types. The presence of TANs in hepatocellular carcinoma, Stage I/II melanoma, and head and neck cancers have been shown to be independently associated with poor overall survival(OS), RFS, and disease-specific survival outcomes(23-25).In contrast, high levels of TANs in stage II colorectal cancer has been associated with improved OS(26). Prior to our study, there has not been a link between tumor associated neutrophil density and recurrence in DTC. Other studies have looked at the peripheral blood neutrophil to lymphocyte ratio (NLR) and found a positive correlation with tumor size among patients with DTC (27-29). Additionally, patients with high American Thyroid Association (ATA) risk for recurrence presented with higher baseline NLR, however, blood NLR was not compared to lymphocytic infiltration within and surrounding the thyroid tumor (27).

There has been debate on the optimal strategy to develop biomarkers for use in early detection, diagnosis, prognosis and assessment of response in cancer (30-33). Tumor infiltrating immune cells and tumor cells in the microenvironment secrete cytokines and

chemokines into circulation to regulate tumor growth. Numerous serum cytokine levels have been studied to understand their role in predicting benign versus malignant thyroid disease (30, 34, 35). Indeed, serum cytokine levels have been previously investigated to see if they correlate with intra-tumor cytokine expression and tumor infiltrating immune cells. In breast cancer, Jabeen et al found that serum cytokine interferon gamma-induced protein 10 (IP10) correlated with mRNA expression in the breast tumor and serum platelet derived growth factor (PDGF) correlated with the tumor immune cells pro-B and natural killer T (NKT) cells (36). Serum Il-2, IL-2R and IL-10 levels have been implicated in distinguishing disease-free patients from those with active disease in thyroid cancer (37). Provatopoulou et al evaluated serum levels of interleukins (IL-1B, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 and IL-13) in a cohort that consisted of 20 papillary thyroid cancer patients, 38 patients with benign nodules and 50 healthy controls using a multiplex assay(35). Peripheral venous blood samples were collected from participants preoperatively. They observed that serum IL-6 and IL-10 concentrations were high in benign and malignant thyroid nodules compared to serum obtained from healthy controls(35). However, none of the individual cytokines were sufficient to discriminate between benign and malignant thyroid nodule. In our study, we investigated the correlation between serum cytokines and tumor infiltrating immune cells in thyroid cancer. We found that tumor with high TAM/low TAN correlated with lower serum levels of the cytokines IL-12p70, Il-8, TNF-alpha and TNF-beta. These cytokines are responsible for a range of signaling events within immune cells (macrophages, neutrophils, T and NK cells) which lead to cellular destruction of tumor cells through processes such as necrosis or apoptosis. We would expect that patients with poor outcomes depicted by lower RFS (high TAM/low TAN tumors) would have associated low levels of these cytokines, produced by the immune cells, in circulation which is what we saw in our study. In our study, patients with low TAM density tumors had significantly higher serum levels of MCP-1, a potent chemoattract that greatly contributes to the recruitment of blood monocytes/macrophage to infiltrate sites of inflammation or tumors. Given this knowledge, we would have expected to see a strong correlation between low TAM density and lower serum levels of MCP-1 and vice versa but this was not the case in our study. It is plausible that we did not see this correlation in our study because the cytokines in circulation may not accurately reflect the intra-tumor cytokines. We acknowledge that this cytokine part of our study is hypothesis-generating, and further work is needed to confirm if circulating cytokines are a true reflection of tumor cytokines in DTC and the potential mechanism for elevation of these cytokines in circulation.

The current study had several limitations that need to be considered when interpreting the results. A major limitation was the relatively low sample size in this analysis. Differentiated thyroid cancer has generally good prognosis and, as such, a larger number of patients with long term follow-up may be necessary to achieve enough power to draw more definitive conclusions regarding the role of tumor immune cell component in predicting outcomes. Cytokine expression in blood was not correlated with intra-tumor cytokine expression and may not completely reflect the changes in the tumor microenvironment. We did not account for previous episodes of fine needle aspiration prior to definitive resection and it is possible that this may affect the density of immune cells present in the resected specimen. Also, we did not have information on background Hashiomoto’s disease in our cohort and as such

cannot conclude if autoimmune disease was a contributing factor to TAM and TAN density. Our finding in the association between high TAM/TAN negative density with lower recurrence-free survival should be interpreted with caution as this is a single institution, observational study. These results should be viewed as hypothesis generating, requiring further validation.

In summary, while scores of TAMs or TANs alone did not predict aggressive histologic features or worse outcomes, when combined, the presence of high TAM density in the absence of TANs is associated with worse outcomes. Additionally, cytokine levels in serum varied by macrophage or neutrophil density present in the tumor microenvironment. Furthermore, assessment of multiple immune cell types in the tumor microenvironment could more accurately predict outcomes in differentiated thyroid cancer. Finally, serum cytokine markers may reflect immune cell density in the tumor microenvironment however further work is needed to confirm this finding.

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Highlights

- Assessment of multiple immune cell types may predict outcomes in thyroid cancer
- High tumor macrophages in low neutrophil density is associated with poor outcome
- Serum cytokine levels may correlate with tumor immune cell composition
- Lower serum levels of IL-12p70, IL8, TNF-α and TNF-β correlated with poor outcome

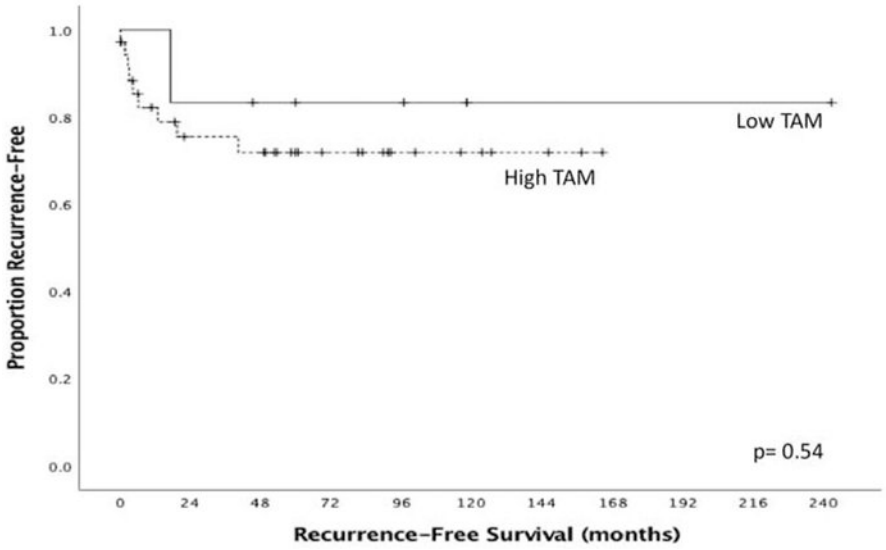

Figure 1:
Recurrence-free survival by TAM density

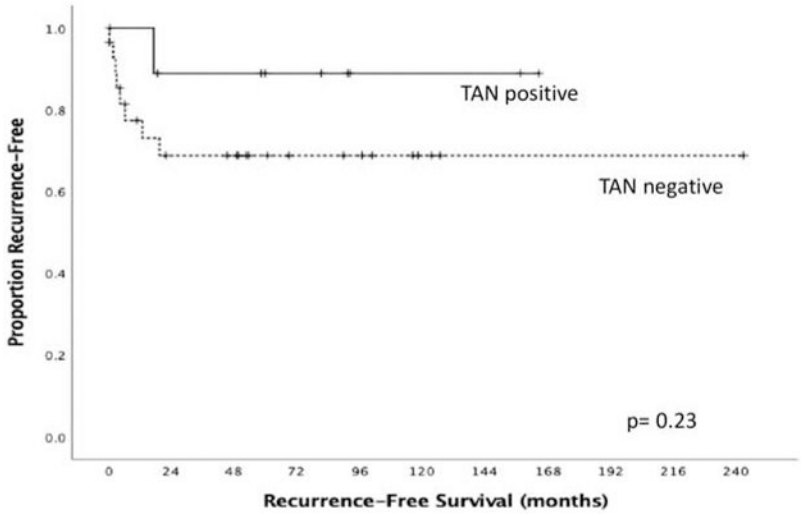

Figure 2:
Recurrence-free survival by TAN density

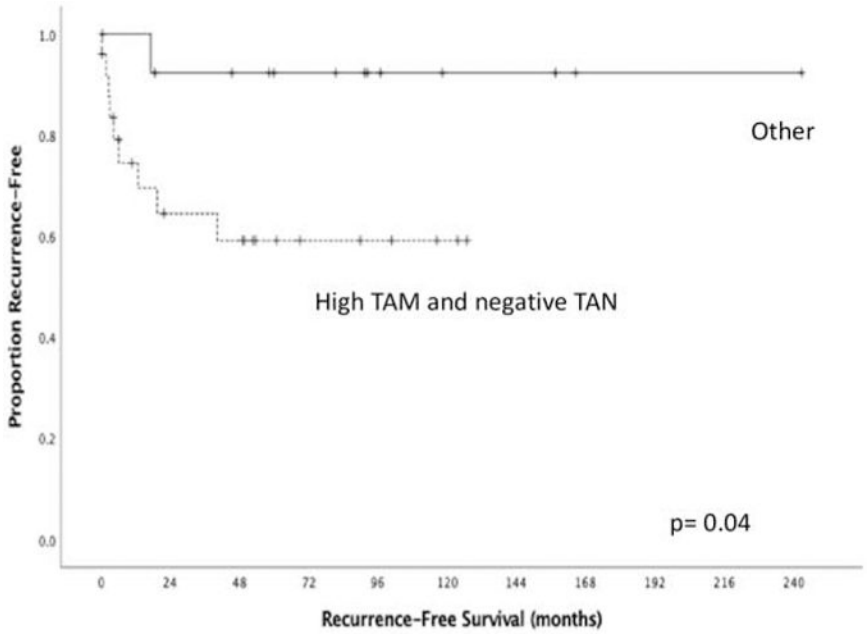

Figure 3:
Patients with high TAM density and negative TANs have significantly lower RFS

Table 1:

Clinicopathologic characteristics of cohort

Patient Characteristics		**N (%)**
Male		14(33.3)
Female		28(66.7)
Age at Diagnosis		
median (range) years		50 (20-86)
Age (Years)	≤ 55 Years	25(59.5)
	> 55 Years	17(40.5)
Cancer Histology	Follicular	8(19.5)
	Papillary	34 (80.9)
	(Classic variant)	25(73.5)
	(Follicular variant)	8(23.5)
	(Oncocytic variant)	1(4.0)
Tumor Size	< 4 cm	33(78.6)
	> 4 cm	8(19.0)
	Unknown	1(2.0)
Capsular Invasion	Yes	9(21.4)
	No	0(0)
	Unknown/NA	33(78.6)
Encapsulated	Follicular	
	Yes	2(25)
	No	6(75)
	Papillary	
	Yes	7(20.6)
	No	27(79.4)
Lymph Node Status at Thyroidectomy (PTC)	Positive	11(26.2)
	Negative	22(52.4)
	Unknown	1(2.0)
Lymphovascular Invasion	Yes	11(26.2)
	No	30(71.4)
	Unknown	1(2.0)
Multifocal tumor	Yes	17(40.5)
	No	24(57.1)
	Unknown	1(2.0)
BRAFV600E positive	Yes	22(52.4)
	No	19(45.2)
	Unknown	1(2.0)

Table 2:

Recurrence-free survival univariable and multivariable analyses

Characteristic	Univariable analysis Hazard ratio (95% confidence interval)	*P* value	Multivariable analysis Hazard ratio (95% confidence interval)	*P* value
Age>55	4.771 (1.225-18.580)	0.02	1.668 (0.357-7.781)	0.52
Female	0.196 (0.050-0.761)	0.02	0.281 (0.065-1.205)	0.09
T stage IV	6.922 (1.852-25.875)	<0.01	5.183 (1.225-21.924)	0.03
Lymph node metastasis	1.874 (0.468-7.501)	0.38	NA	NA
Multifocal tumors	1.781 (0.478-6.634)	0.39	NA	NA
Lymphovascular invasion	2.823 (0.756-10.537)	0.12	NA	NA
Capsular invasion	1.625 (0.388-6.813)	0.51	NA	NA
BRAFV600E	1.481 (0.299-7.348)	0.63	NA	NA
TAN density	0.302 (0.038-2.417)	0.23	NA	NA
TAM density	1.891 (0.239-14.946)	0.54	NA	NA

Table 3:

Correlating tumor associated macrophages with clinicopathologic characteristics

Clinicopathologic Characteristics	Low TAM	High TAM	p-value
Age >55	16.7%	44.4%	0.20
Female gender	66.7%	61.1%	0.80
T stage IV	0%	25%	0.23
Lymph node metastasis present	50%	48.6%	0.95
Multifocal tumors present	33.3%	42.9%	0.66
Lymphovascular invasion present	33.3%	25.7%	0.70
Capsular invasion present	50%	21.2%	0.21
BRAFV600E positive	50%	69%	0.45

Table 4:

Comparison of serum cytokine levels with tumor associated macrophage expression

	Low TAM N=24	High TAM N=18	p-value
Eotaxin	4.17 (3.95-4.34)	4.08 (3.86-4.23)	0.50
MIP1a	2.57 (2.52-2.70)	2.69 (2.63-2.83)	0.03
IL16	4.19 (4.09-4.48)	4.24 (4.08-4.59)	0.99
VEGF	3.60 (3.48-3.85)	3.55 (3.39-3.83)	0.79
IL2	2.67 (2.63-2.69)	2.66 (2.64-2.69)	0.85
Eotaxin3	2.51 (2.39-2.58)	2.52 (2.42-2.56)	0.96
MIP1b	4.08 (3.92-4.15)	4.08 (4.00-4.20)	0.54
IL17	2.59 (2.50-2.68)	2.54 (2.50-2.59)	0.52
IFNgamma	3.02 (2.92-3.14)	2.94 (2.86-3.16)	0.50
IL4	2.35 (2.32-2.38)	2.37 (2.32-2.46)	0.26
IL8	2.21 (2.19-2.26)	2.26 (2.23-2.28)	0.01
TARC	4.04 (3.91-4.23)	4.00 (3.83-4.12)	0.29
IL1a	2.59 (2.54-2.64)	2.56 (2.50-2.63)	0.29
IL10	2.74 (2.71-2.86)	2.74 (2.72-2.76)	0.81
IL6	2.69 (2.56-2.88)	2.70 (2.51-2.96)	0.93
IP10	4.87 (4.79-5.02)	4.80 (4.64-4.92)	0.16
GMCSF	2.32 (2.30-2.37)	2.34 (2.30-2.36)	0.88
IL5	2.73 (2.70-2.78)	2.75 (2.69-2.84)	0.31
IL12p70	2.47 (2.43-2.53)	2.47 (2.44-2.56)	0.56
AE	3.38 (3.30-3.64)	3.55 (3.38-3.93)	0.14
MCP1	4.75 (4.66-4.98)	4.64 (4.55-4.79)	0.01
IL12	4.18 (4.03-4.32)	4.16 (4.09-4.41)	0.77
IL7	2.62 (2.56-2.93)	2.62 (2.55-2.77)	0.38
IL13	2.30 (2.28-2.32)	2.34 (2.29-2.44)	0.08
TNFalpha	3.14 (3.07-3.26)	3.14 (3.06-3.31)	0.89
MDC	4.33 (4.22-4.40)	4.33 (4.25-4.46)	0.77
IL15	2.91 (2.89-2.99)	2.91 (2.84-2.94)	0.36
TNFbeta	2.59 (2.55-2.67)	2.61 (2.56-2.64)	0.74
IL1b	2.93 (2.85-3.19)	2.92 (2.83-3.63)	0.79
RANTES	4.22 (3.99-4.98)	4.15 (4.01-4.42)	0.57

Data are presented as log transformed values. Median (interquartile range)

Table 5:

Correlating tumor associated neutrophils with clinicopathologic characteristics

Clinicopathologic Characteristics	TAN positive	TAN negative	p-value
Age >55	10%	50%	0.03
Female gender	50%	90%	0.03
T stage IV	0%	18.5%	0.14
Lymph node metastasis present	30%	51.9%	0.24
Multifocal tumors present	30%	44.4%	0.43
Lymphovascular invasion present	20%	33.3%	0.43
Capsular invasion present	33.3%	16.7%	0.30
BRAFV600E positive	70%	63.2%	0.71

Table 6:

Comparison of serum cytokine levels with tumor associated neutrophil expression

	TAN Negative N=28	TAN positive N=10	p-value
Eotaxin	4.16 (3.97-4.34)	4.20 (3.76-4.34)	0.89
MIP1a	2.68 (2.52-2.80)	2.64 (2.56-2.77)	0.86
IL16	4.24 (4.09-4.59)	4.14 (4.06-4.56)	0.65
VEGF	3.62 (3.39-3.85)	3.60 (3.47-3.72)	0.93
IL2	2.67 (2.63-2.70)	2.66 (2.65-2.69)	0.87
Eotaxin3	2.52 (2.42-2.59)	2.49 (2.40-2.55)	0.51
MIP1b	4.08 (3.92-4.20)	4.06 (4.00-4.13)	0.82
IL17	2.55 (2.49-2.62)	2.57 (2.54-2.67)	0.50
IFNgamma	3.02 (2.92-3.14)	3.00 (2.84-3.18)	0.72
IL4	2.37 (2.32-2.39)	2.36 (2.34-2.42)	0.65
IL8	2.26 (2.21-2.26)	2.22 (2.18-2.26)	0.26
TARC	4.01 (3.91-4.22)	4.01 (3.76-4.39)	0.93
IL1a	2.59 (2.54-2.64)	2.56 (2.51-2.63)	0.57
IL10	2.74 (2.72-2.86)	2.74 (2.72-2.76)	0.33
IL6	2.73 (2.54-2.90)	2.64 (2.55-2.81)	0.50
IP10	4.86 (4.79-5.05)	4.83 (4.71-4.95)	0.47
GMCSF	2.33 (2.30-2.37)	2.33 (2.31-2.35)	0.50
IL5	2.75 (2.71-2.79)	2.72 (2.70-2.81)	0.68
IL12p70	2.50 (2.45-2.56)	2.42 (2.41-2.47)	0.01
MCP1	4.75 (4.64-4.82)	4.76 (4.60-4.92)	0.89
IL12	4.18 (4.03-4.35)	4.15 (4.04-4.36)	0.93
IL7	2.61 (2.56-2.78)	2.66 (2.49-2.91)	0.93
IL13	2.30 (2.29-2.35)	2.29 (2.28-2.32)	0.47
TNFalpha	3.14 (3.07-3.31)	3.12 (3.08-3.16)	0.16
MDC	4.33 (4.22-4.36)	4.35 (4.29-4.45)	0.37
IL15	2.91 (2.85-2.99)	2.90 (2.82-2.94)	0.38
TNFbeta	2.62 (2.57-2.69)	2.59 (2.54-2.60)	0.10
IL1b	2.91 (2.85-3.34)	2.94 (2.86-3.24)	0.89
RANTES	4.09 (3.98-4.58)	4.28 (4.11-4.73)	0.39

Data are presented as log transformed values. Median (interquartile range)

Table 7:

Comparison of combined High TAM/Negative TAN with serum cytokines.

	Other N=20	High TAM Negative TAN N=11	p-value
IP10	4.89 (4.80-5.08)	4.80 (4.68-4.92)	0.17
MIP1beta	4.10 (3.93-4.24)	4.07 (4.00-4.13)	0.51
IL15	2.91 (2.85-2.99)	2.90 (2.85-2.94)	0.26
IL5	2.75 (2.72-2.79)	2.72 (2.69-2.79)	0.48
IFNgamma	3.02 (2.92-3.15)	2.93 (2.85-3.11)	0.41
IL1beta	2.90 (2.85-3.34)	2.95 (2.85-3.34)	1.00
IL8	3.42 (3.21-3.70)	3.38 (3.38-3.44)	0.92
Eotaxin	4.12 (3.97-4.39)	4.17 (3.90-4.29)	0.97
MCP1	4.76 (4.64-4.83)	4.74 (4.61-4.83)	0.74
TARC	4.02 (3.87-4.19)	3.99 (3.79-4.33)	0.84
IL16	4.24 (4.11-4.60)	4.15 (4.05-4.43)	0.56
IL7	2.61 (2.56-2.77)	2.62 (2.49-3.00)	0.84
IL10	2.76 (2.73-2.86)	2.73 (2.70-2.75)	0.06
IL2	2.67 (2.63-2.70)	2.67 (2.65-2.68)	0.98
TNFalpha	3.17 (3.13-3.34)	3.10 (3.06-3.14)	0.01
Eotaxin3	2.53 (2.41-2.61)	2.51 (2.41-2.56)	0.69
MDC	4.33 (4.25-4.38)	4.34 (4.23-4.43)	0.93
GMCSF	2.34 (2.30-2.38)	2.32 (2.29-2.34)	0.13
IL17	2.57 (2.50-2.65)	2.54 (2.50-2.66)	0.54
TNFbeta	2.63 (2.58-2.69)	2.58 (2.53-2.60)	0.01
IL12p70	2.51 (2.47-2.56)	2.43 (2.41-2.50)	0.01
IL4	2.37 (2.32-2.40)	2.36 (2.34-2.38)	0.90
RANTES	4.10 (3.97-4.50)	4.23 (4.05-5.04)	0.34
IL8	2.26 (2.21-2.28)	2.21 (2.19-2.25)	0.02
MIP1alpha	2.70 (2.55-2.81)	2.58 (2.49-2.68)	0.16
IL12	4.22 (4.04-4.41)	4.12 (3.97-4.32)	0.25
IL1alpha	2.58 (2.54-2.64)	2.56 (2.51-2.63)	0.71
VEGF	3.58 (3.43-3.83)	3.60 (3.39-3.85)	0.90
IL13	2.30 (2.29-2.36)	2.29 (2.28-2.34)	0.30
IL6	2.80 (2.64-2.95)	2.59 (2.53-2.69)	0.15

Data are presented as log transformed values. Median (IQR).