Prognostic Immune Markers in Non-Small Cell Lung Cancer

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Statement of Translational Relevance

Recurrence in lung cancer continues to pose a major clinical challenge, with rates of recurrence as high as 40% among early stage patients who undergo resection with curative intent. This highlights the need for additional prognostic markers and therapeutic targets. The host immune response to tumor is a critical component in tumor progression and a prognostic marker in colorectal and ovarian cancers. In this first comprehensive review of immune markers of non-small cell lung cancer (NSCLC) in (a) the tumor microenvironment, (b) peripheral blood, and (c) gene profiling studies, we identify those immune markers that are prognostic. This review provides a foundation for future studies investigating tumor immunology in NSCLC.
Abstract

Tumor-associated immune responses have polarized effects in regulating tumor growth. While a clear association has been shown between the tumor immune response and clinical outcome in colorectal and ovarian cancers, the role of immune markers for stratifying prognosis in non-small cell lung cancer (NSCLC) is less defined. Herein we review the prognostic significance of published immune markers in the tumor microenvironment as well as peripheral blood of NSCLC patients. To identify prognostic immune genes, we reviewed all published gene profiling studies in NSCLC and delineated the significance of immune genes by performing subanalysis on the microarray database of the NIH Director’s Challenge study. This first comprehensive review of prognostic immune markers provides a foundation for further investigating immune responses in NSCLC.
Introduction

Immune responses within the tumor microenvironment are increasingly implicated as a determining factor in tumor progression and aggressiveness. Understanding these responses has allowed investigators to use immune markers to stratify prognosis of colorectal and ovarian cancer patients (1, 2). High levels of intratumoral effector memory cells in colorectal cancer and CD3+ T cells in ovarian cancer are associated with prolonged survival.

Published studies investigating immune markers as prognosticators in non-small cell lung cancer (NSCLC) are heterogeneous in histology (adenocarcinoma, squamous cell, and large cell) and stage (I-IV). In this review, we have identified prognostic immune markers in the tumor microenvironment and peripheral blood, as well as tumor expression of immune genes. The immune genes, from 17 published NSCLC gene profiling studies, were evaluated for prognostic significance in the NIH Director’s Challenge microarray (3), the largest multi-center gene profiling study to date.

Prognostic Immune Cells

Tumor-Infiltrating Lymphocytes (TILs)

The interaction between tumor and immune cells in the tumor microenvironment is influenced by the type of immune cells (CD8+, CD4+, CD20+, and FoxP3+ - see Table 1), their density (as counted microscopically), and location (tumor nest and stroma) (4). Among T lymphocytes, which comprise 80% of TILs in NSCLC (5), CD8+ cytotoxic lymphocytes...
form the effector arm of adaptive immunity and are thought to have protective roles against tumors. However, five investigations in NSCLC show no correlation between CD8+ TIL infiltration and survival (6-10). Pertinent to NSCLC patients is the fact that CD8+ T-cell predominance is a characteristic of smoking-related chronic obstructive pulmonary disease (11), and they are thought to drive the progression of emphysema (12). In mouse models, activation of CD8+ T cells are impaired in the presence of cigarette smoke (13, 14), perhaps explaining this finding in NSCLC patients. In fact, studies that report correlation between CD8+ TIL and prognosis contradict each other (15, 16). Ruffini et al. demonstrate in their investigation of 1290 NSCLC tumors that TILs, mostly CD8+, were associated with prolonged survival but only in a subset of squamous cell carcinomas (n=549) (15). This is contrasted by Wakabayashi et al., who correlate tumoral CD8+ and shorter overall survival in NSCLC, especially in adenocarcinoma (16). Examining the functional significance of CD8+ T-cells, Trojan et al. analyzed mRNA ratio of IFN-γ to CD8, where IFN-γ is an effector cytokine secreted by cytotoxic T cells, specifically in peri-tumoral areas and within tumor nests. While the number of peritumoral CD8+ T cells correlated with the IFN-γ/CD8 ratio, this association was not observed intra-tumorally (8). This suggests that while the CD8+ T-cells are able to infiltrate the tumor, they are not able to mount a robust anti-tumor response once within the tumor nest.

In contrast to CD8+ lymphocytes, stromal CD4+ (16, 17), CD20+ (17, 18), and
co-localization of stromal CD8+ and CD4+ cells (9) have all shown association with improved survival. The significance of immune cell co-localization is highlighted in a study by Dieu-Nosjean et al., who demonstrate the presence of tertiary de novo lymphoid structure in the tumor microenvironment, which they named the tumor-induced bronchus-associated lymphoid tissue (Ti-BALT) (19). In their investigation of 74 stage I-IIA NSCLC, the presence of mature dendritic cells (Lamp+, lysosome-associated membrane protein) in Ti-BALT correlate with higher density of T and B lymphocytes and more importantly prolonged overall (OS) and disease-free survival (DFS) (4-year DFS of 51% vs 88%). The investigators postulate that effector immune cell co-localization within Ti-BALT could play a role in activating an anti-tumor immune response.

**Regulatory T-cells (Tregs)**

Regulatory T cells (Tregs) suppress the host immune response and are thought to promote tumor growth. The pro-tumor association of Treg tumor infiltration was examined by Shimizu et al. in a study of stage I-III 100 NSCLC tumors, demonstrating that tumor-infiltrating FoxP3+ Tregs correlate with cyclo-oxygenase-2 (COX-2) expression and increased tumor recurrence (20). This is supported by preclinical findings from Sharma et al., who demonstrated that tumor-derived COX-2 and its product prostaglandin E_2 (PGE_2) induced *in vitro* lymphocyte expression of FoxP3. *In vivo*, inhibition of COX-2 reduced Treg activity, attenuated FoxP3 expression in TILs, and decreased tumor burden (21). Furthermore,
urinary PGE-M, the major metabolite of PGE\(_2\), was proposed as a biomarker to predict response to COX-2 inhibitors in NSCLC patients (22). Petersen et al. demonstrated that Treg/TIL Combination Risk Index (the ratio of FoxP3\(^+\) to CD3\(^+\)) correlates with disease-specific survival (DSS) in patients with stage I NSCLC (23). Patients with high-risk tumors (30% of tumors) experienced worse DSS (median 53 months) when compared to patients with intermediate (63 months) and low-risk tumors (>72 months), which had low FoxP3 and high CD3.

**Tumor-Associated Macrophages (TAMs)**

TAMs demonstrate both pro- and anti-tumor effects in the tumor microenvironment (24). Anti-tumor TAMs in NSCLC are of the M1 phenotype and accumulate intra-tumorally while pro-tumor TAMs of the M2 phenotype accumulate in the stroma and express IL-8, IL-10, and triggering receptor expressed on myeloid cells (TREM-1). IL-8 is an angiogenic factor, and TAM’s angiogenic role in NSCLC has been shown by correlating macrophage density with intra-tumor microvessel counts and poor patient outcomes (25). By performing RT-PCR for IL-8 in resected specimens, the study concludes that TAMs lead to poor patient outcome by their angiogenic role through IL-8 production. IL-10 is an immunosuppressive cytokine, and its expression by TAM has been observed more commonly in stages II/III/IV, thus correlating with decreased OS (26). TAM expression of TREM-1, which can initiate and amplify an inflammatory response, is increased in malignant pleural effusions of NSCLC.
Furthermore, in 68 stage I-III NSCLC patients, increased level of TREM-1 high TAMs in resected specimen was an independent predictor of shorter OS.

In contrast to these findings, TAMs have also been associated with prolonged survival in a study of 144 stage I-IV NSCLC (28). Recent studies have shed light on these contradictory findings, where TAMs have been classified according to their location and phenotype. In an investigation 175 stage I-III NSCLC tumors, Welsh et al. found that CD68+ macrophages in the stroma are associated with poor prognosis (5-year OS of 27% vs 35%) while intra-tumoral macrophages are associated with increased survival (5-year OS of 53% vs 8%) (29).

Phenotypically, TAMs are comprised of 2 distinct types: M1, pro-inflammatory with anti-tumor activity, and M2, which are immunosuppressive, angiogenic, and pro-tumor (30, 31). In a nested case-control study, 20 long-survival patients (median 93 months) had high tumor infiltration of the M1 phenotype compared to 20 poor-survival patients (median 8 months). M1 was characterized by the expression of human leukocyte antigen (HLA)-DR, inducible nitric oxide synthase (iNOS), myeloid related protein (MRP) 8/14, and tumor necrosis factor (TNF)-α (32). Contrasting the anti-tumor association of M1 TAMs, high numbers of CD204+ M2 TAMs in stroma have been shown to correlate with decreased OS (33). CD204+ TAMs are also associated with increased tumor expression of IL-10 and monocyte chemoattractant protein (MCP)-1, two molecules which are known to attract
macrophages.

**Tumor-Associated Neutrophils (TANs)**

Similar to TAMs, TANs also have polarized functions. Although no published studies investigate the role of TAN in human NSCLC, recent preclinical work by Fridlender et al. showed that in mouse models of lung cancer, TGF-β induces a population of TAN with pro-tumor function (N2) while TGF-β blockade results in anti-tumor neutrophils (N1) (34). Depletion of these N1 neutrophils resulted in increased tumor growth.

**Peripheral Blood Lymphocytes**

The universal availability of peripheral blood lymphocyte count has led to its investigation as a prognostic marker in NSCLC. In resected NSCLC patients (n=177; 72% Stages I and II), increasing total lymphocyte counts were associated with lower hazard ratios for death (HR 0.62, CI=0.43-0.9; p=0.012) (35). The same study further identified neutrophil to lymphocyte ratio (NLR) as a superior predictor of survival compared to pathologic stage (p=0.001) (35). Specifically, an NLR of >3.81 was found to be a significant predictor of survival in patients with stage I NSCLC. As for the tumor-promoting Tregs, they have been observed at an increased level in the peripheral blood of NSCLC patients compared to normal healthy volunteers (36, 37). These reports correlate with elevated serum and plasma levels of TGF-β and IL-10, both known promoters of Treg development, in NSCLC patients compared
to healthy controls (38, 39). Peripheral blood levels of Tregs, TGF-β, and IL-10, however, have not been investigated in predicting clinical outcome of NSCLC patients.

**Myeloid-Derived Suppressor Cells (MDSCs):**

MDSCs represent a heterogeneous population of myeloid cells comprising immature macrophages, granulocytes, and DCs at early stages of differentiation that have pro-tumor effects. MDSCs are mobilized from bone marrow into the peripheral blood by tumor-derived factors and accumulate in the tumor microenvironment, where they exert their pro-tumor effect by inhibiting T cell proliferation and activation (40). Similar to Tregs, increased levels of MDSCs, marked by CD11b+/CD14-/CD15+/CD33+, were observed in the peripheral blood of advanced stage NSCLC patients (n = 87) compared to healthy controls (41). In addition, high levels of MDSCs were associated with decreased levels of CD8+ T cells, further supporting the pro-tumor effect of MDSCs. No study to date, however, has investigated the prognostic significance of MDSCs in the tumor microenvironment of patient samples.

**Summary of Prognostic Tumor-Infiltrating Immune Cells**

Stromal CD4+ lymphocytes, especially co-localized with CD8+ lymphocytes, stromal CD20+ B lymphocytes, Ti-BALT, and intra-tumoral macrophages (M1) are associated with prolonged survival in NSCLC. In contrast, FoxP3+ Tregs, stromal macrophages (M2), and their associated cytokines – IL-8, IL-10, and TREM-1 – are associated with poor prognosis.
Tumor expression and secretion of COX-2 and IL-10, respectively, lead to shorter survival.

These prognostically significant findings are summarized in Figure 1.

**Prognostic Immune Genes**

In the large, multi-site, blinded validation study examining lung adenocarcinoma (NIH Director’s Challenge), one of the four prognostic gene clusters contained genes related to immunologic function (3). Further pathway analysis demonstrated immune function to be important in stratifying lung adenocarcinomas into prognostic subgroups (42). In addition to the NIH Director’s Challenge study, we reviewed all published genomic studies performed on lung cancer between 2000-2010 that utilized mRNA microarray or RT-PCR. From the 17 studies reviewed (total of 1,615 patients, **Supplementary Table 1**) (3, 43-58), we identified the prognostic genes related to immune/inflammatory response (search/selection criteria shown in **Supplementary Figure 1**), resulting in a list of 84 genes (**Supplementary Table 2**).

As shown in **Figure 2**, there are seven overlapping immune genes from the 17 reviewed studies, and two genes are from a well-investigated chemokine axis in NSCLC – CCL19/CCR7. CCR7 (C-C chemokine receptor type 7) is a chemokine receptor expressed on naïve T cells, dendritic cells, natural killer cells, and B cells, and its interaction with its ligands, CCL19 (C-C motif chemokine 19) and CCL21 (C-C motif chemokine 21), play a central role in lymphocyte trafficking and homing to lymph nodes (59, 60). In lung cancer,
CCR7 expression on tumor cells by mRNA has been demonstrated as an independent predictor of lymph node metastasis in an investigation of 71 NSCLC patients (61). It has been hypothesized that when expressed on tumors cells, the homing mechanism of CCR7 contributes to tumor’s increased potential for lymphatic metastasis.

While CCR7 may increase the metastatic potential of tumor cells, investigators have attempted to exploit the effect of this chemokine axis on the immune cells to promote tumor regression. CCL19, or Epstein-Barr virus-induced molecule 1 ligand chemokine (ELC), expressed in the T cell zone of lymph nodes and dendritic cells, is known to attract immune cells which express CCR7. While no clinical study has investigated the role of CCL19 in NSCLC, its ability to promote tumor infiltration of T cells and dendritic cells (DCs) has been shown in preclinical models to decrease tumor burden. Intra-tumoral injection of CCL19 lead to increased influx of CD4+ and CD8+ T cells and DCs, increase in anti-tumor factors such as IFN-γ and IL-12, and a decrease in the immunosuppressive molecule TGF-β (62, 63).

CCL21, the other ligand of CCR7, has been investigated as a potential adjunct to DC-based therapy due to its ability in promoting co-localized tumor infiltration of DCs and lymphocyte effector cells (64). Intratumoral injection of DCs transduced to express CCL21 reduced tumor burden in a mouse model of spontaneous bronchoalveolar carcinoma. Based on these findings, a phase I clinical trial is currently investigating the intratumoral injection of CCL-21-expressing DCs in stage IIIb/IV NSCLC patients refractory to standard therapy
Significance of Prognostic Immune Genes in the NIH Director's Challenge study

Cox proportional hazards model was used to evaluate the association between DFS and the expression profiles of the 84 identified prognostic immune genes in the Director’s Challenge dataset (3). This analysis revealed 17 genes which were significantly associated with recurrence (Table 2). We employed the Ingenuity Pathway Analysis to examine the networks shared by the 17 genes (Figure 3). STAT3 was a common signal transduction pathway highlighted in this analysis, which has been implicated in cancer inflammation and immunity (66).

Although prognostic immune genes are identified in NSCLC, the cells of origin (tumor cells, tumor-infiltrating immune cells, or both) of these genes remain to be explored. One of the 17 prognostic genes, IL-7R, is expressed by both the tumor and immune cells. When expressed on the tumor, IL-7R expression leads to up-regulation of VEGF-D in vitro and also shorter OS in NSCLC patients (67). On the other hand, it has been demonstrated in vitro that the expression of IL-7R on CD8+ T cells signifies a long-lived memory phenotype (68). How the expression of these genes modulates the tumor immune microenvironment is future area of investigation. CXCR4 is the receptor for stromal-derived factor-1alpha (CXCL12). In lung adenocarcinoma, tumor expression of CXCL12 has been shown to correlate with accumulation of CXCR4-expressing immune cells, 30% of which were
pro-tumor regulatory T cells (69).

**Conclusion**

The immune microenvironment surrounding the tumor is influenced by interactions among tumor, immune cells (TILs, TAMs, TANs, and MSDCs), and cytokines that shift the environment to pro- or anti-tumor. In NSCLC, stromal CD4+ T lymphocytes, especially co-localized with CD8+ T cells, stromal CD20+ B lymphocytes, Ti-BALT, and intra-tumoral M1 macrophages predicted prolonged patient survival. FoxP3+ Tregs and stromal M2 macrophages are associated with poor clinical outcome. We examined gene expression profiling studies for insights into the NSCLC tumor microenvironment. In reviewing 17 gene profile studies, we identified 17 prognostic immune genes by comparing their expression to tumor recurrence in lung adenocarcinoma patients. Further studies utilizing RT-PCR and immunohistochemical analysis are necessary to determine the source cell (tumor versus immune) and how they influence the tumor immune microenvironment. Future studies investigating a large patient series uniform in histology and stage and assessing tumor-immune interaction in both tumor nest and tumor-associated stroma may yield significant information.

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References:


Figure Legends:

**Figure 1. Prognostic immune markers in NSCLC.** T cells and B cells are associated with longer survival when found in the stroma along with Ti-BALT which contains Lamp+ dendritic cells. In contrast, FoxP3+ Tregs in the tumor are associated with shorter survival. Anti-tumor M1 macrophages are characterized by HLA-DR, iNOS, MRP, and TNF-α. Pro-tumor M2 macrophages express CD204. M2 expression of IL-8, IL-10, and TREM-1 (delineated by arrows) has been shown to correlate with shorter survival. Tumoral expression of COX-2 recruits FoxP3+ Tregs cells while expression of IL-10 and MCP-1 recruits M2 macrophages. In the peripheral blood, immune suppression is associated with poor clinical outcomes revealed by low TLCs and elevated NLRs.

COX, cyclooxygenase; DC, dendritic cells; FoxP3, forkhead box P3; IL, interleukn; iNOS, inducible nitric oxide synthase; LAMP, lysosome-associated membrane protein; MCP, monocyte chemoattractant protein; MRP, myeloid-related protein; NLR, neutrophil to lymphocyte ratio; Ti-BALT, tumor-induced bronchus-associated lymphoid tissue; TLC, total lymphocyte count; TNF, tumor necrosis factor; TREM, triggering receptor expressed on myeloid cells; Treg, regulatory T cells.
**Figure 2. Prognostic genes associated with immunity and inflammation.** Prognostic immune/inflammatory genes from published gene profile studies on lung cancer, with overlapping immune genes shown in boxes.

**Figure 3. Ingenuity Pathway Analysis of 17 significant immune genes.** The data set containing gene identifiers was uploaded into the application. Each identifier was mapped to its corresponding object in Ingenuity's Knowledge Base. A P value cutoff of 0.05 was set to identify molecules whose expression was significantly differentially regulated. These molecules, called Network Eligible molecules, were overlaid onto a global molecular network developed from information contained in Ingenuity's Knowledge Base. Networks of Network Eligible Molecules were then algorithmically generated based on their connectivity.
Supplementary Figures:

Supplementary Figure 1. Selection criteria for reviewed studies on gene expression profiles and approach to identification of prognostic immune genes.

Initial search for this review was performed by searches of PubMed and references from relevant articles from using the search terms “gene expression profiling” and “lung cancer”.

# Table 1 Summary of TIL and TAM role in lung cancer with respect to their phenotype and location *

<table>
<thead>
<tr>
<th>Author</th>
<th># pts</th>
<th>stages</th>
<th>Observation/Conclusion</th>
<th>Survival Advantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Johnson</td>
<td>95</td>
<td>I- 54</td>
<td>(57%) High CD3+ and S100+ in tumor correlated with longer OS</td>
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<tr>
<td></td>
<td></td>
<td>II- 17</td>
<td>(18%)</td>
<td></td>
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<td></td>
<td></td>
<td>III 20</td>
<td>(21%)</td>
<td></td>
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<tr>
<td>Hiraoka</td>
<td>109</td>
<td>I -67</td>
<td>(61%) Concurrent high CD4+ and CD8+ in stroma correlated with longer survival</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>II-II- 42</td>
<td>(39%)</td>
<td></td>
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<tr>
<td>Kikuchi</td>
<td>161</td>
<td>I – 95</td>
<td>(59%) HLA class I expression correlates with longer OS in stage I</td>
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<tr>
<td></td>
<td></td>
<td>II-IV - 66</td>
<td>(41%)</td>
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<tr>
<td></td>
<td></td>
<td>I - 714</td>
<td>(55%)</td>
<td></td>
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<tr>
<td>Ruffini</td>
<td>1290</td>
<td>II-265</td>
<td>(21%) TIL (mostly CD8+) in tumor correlated with better OS</td>
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<tr>
<td></td>
<td></td>
<td>IIIA -214</td>
<td>(17%)</td>
<td></td>
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<td></td>
<td></td>
<td>I -107</td>
<td>(60%) High CD4+ in stroma correlated with longer OS</td>
<td>5-yr OS 64% vs 43%</td>
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<td></td>
<td></td>
<td>IIIA -48</td>
<td>(27%)</td>
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<tr>
<td>Wakabayashi</td>
<td>178</td>
<td>II-23</td>
<td>(13%) High CD8+ in tumor correlated with shorter OS</td>
<td>5-yr OS 47% vs 60%</td>
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<td></td>
<td></td>
<td>IIIA -48</td>
<td>(27%)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>I -212</td>
<td>(63%) High CD4+ in stroma correlated with longer DSS</td>
<td>5-yr DSS 63% vs 42%</td>
</tr>
<tr>
<td>Al-Shibli</td>
<td>335</td>
<td>II - 91</td>
<td>(27%) High CD8+ in stroma correlated with longer DSS</td>
<td>5-yr DSS 75% vs 53%</td>
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<tr>
<td></td>
<td></td>
<td>IIIA -32</td>
<td>(10%)</td>
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<td></td>
<td></td>
<td>I-66</td>
<td>(58%) High CD20+ in stroma correlated with longer DSS</td>
<td>5-yr DSS 61% vs 32%</td>
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<tr>
<td>Pelletier</td>
<td>113</td>
<td>II - 20</td>
<td>(18%) Peritumoral CD20+ correlated with longer survival</td>
<td></td>
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<td></td>
<td></td>
<td>III -29</td>
<td>(26%)</td>
<td></td>
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<tr>
<td>Dieu-Nosjean</td>
<td>74</td>
<td>I - 62</td>
<td>(84%) High mature dendritic cells in tertiary lymphoid structures correlated with longer survival</td>
<td>4-yr DFS 88% vs 51%</td>
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<tr>
<td></td>
<td></td>
<td>IIA -12</td>
<td>(16%)</td>
<td></td>
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<tr>
<td>Shimizu</td>
<td>100</td>
<td>I – 68</td>
<td>(68%) High FoxP3+ correlated with shorter time to recurrence</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>II – 14</td>
<td>(14%) COX-2 expression correlated with shorter time to recurrence</td>
<td></td>
</tr>
<tr>
<td>Petersen</td>
<td>64</td>
<td>I - 64</td>
<td>High proportion of FoxP3+ among TIL in tumor correlated with shorter DFS</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>II-23</td>
<td>(13%) High FoxP3+ correlated with shorter time to recurrence</td>
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<td></td>
<td></td>
<td>IIIA -48</td>
<td>(27%)</td>
<td></td>
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<tr>
<td>Chen</td>
<td>35</td>
<td>I - 14</td>
<td>(40%) TAM in stroma correlated with shorter OS</td>
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<td></td>
<td></td>
<td>II - 4</td>
<td>(11%) TAM-tumor interaction upregulated IL-8 mRNA expression</td>
<td>median OS 16 mo vs 45 mo</td>
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<td></td>
<td>III - 17</td>
<td>(49%)</td>
<td></td>
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<tr>
<td>Zeni</td>
<td>47</td>
<td>I – 24</td>
<td>(51%) IL-10 high TAMS associated with shorter OS</td>
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<td></td>
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<td>II/II-IV - 23</td>
<td>(49%)</td>
<td></td>
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<td></td>
<td></td>
<td>I –24</td>
<td>(35%) IL-10 high TAMS associated with advanced stage</td>
<td></td>
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<tr>
<td>Ho</td>
<td>68</td>
<td>II – 15</td>
<td>(22%) Increased high TREM-1 macrophages correlated with shorter DFS and OS</td>
<td>Median DFS 22 mo vs not reached</td>
</tr>
<tr>
<td></td>
<td></td>
<td>III – 29</td>
<td>(43%)</td>
<td>Median DFS 29 mo vs not reached</td>
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<td></td>
<td>I - 79</td>
<td>(55%)</td>
<td></td>
</tr>
<tr>
<td>Kim</td>
<td>144</td>
<td>II - 25</td>
<td>(17%) TAM in tumor correlated with longer OS</td>
<td>5-yr OS 64% vs 39%</td>
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<td></td>
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<td>III - 38</td>
<td>(26%)</td>
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<td></td>
<td></td>
<td>I - 79</td>
<td>(45%)</td>
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<tr>
<td>Welsh</td>
<td>175</td>
<td>II - 44</td>
<td>(25%) High TAM in tumor correlated with longer OS</td>
<td>5-yr OS of 53% vs 8%</td>
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<td></td>
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<td>IIIA - 34</td>
<td>(19%)</td>
<td></td>
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<td></td>
<td>I – 26</td>
<td>(65%)</td>
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<tr>
<td>Ohri</td>
<td>40</td>
<td>II – 8</td>
<td>(20%) Increased M1 in long survivors</td>
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<td></td>
<td></td>
<td>III – 6</td>
<td>(15%)</td>
<td></td>
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<tr>
<td>Ohtaki</td>
<td>170</td>
<td>IA – 95</td>
<td>(56%) High stromal CD204+ (M2) associated with shorter survival</td>
<td>5-yr OS of 61% vs 89%</td>
</tr>
<tr>
<td></td>
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<td>IB-IIIA – 75</td>
<td>(44%)</td>
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<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>p.value (HR)</th>
<th>Gene Name</th>
<th>Function</th>
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<tbody>
<tr>
<td>LIME1 (20q13.3)</td>
<td>0.0007 (1.0036)</td>
<td>LCK interacting transmembrane adaptor 1</td>
<td>Involved in BCR (B-cell antigen receptor)-mediated signaling in B-cells</td>
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<td></td>
<td></td>
<td>Involved in TCR (T-cell antigen receptor)-mediated T-cell signaling in T-cells</td>
</tr>
<tr>
<td>IL7R (5p13)</td>
<td>0.0012 (0.9993)</td>
<td>interleukin-7 receptor subunit alpha; CD127</td>
<td>Receptor for interleukin-7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Expressed at high levels on naïve T cells</td>
</tr>
<tr>
<td>CD59 (11p13)</td>
<td>0.0034 (0.9997)</td>
<td>CD59 glycoprotein membrane attack complex inhibitory protein</td>
<td>Potent inhibitor of the complement membrane attack complex (MAC) action</td>
</tr>
<tr>
<td>RHOH (4p13)</td>
<td>0.0102 (0.9977)</td>
<td>Rho-related GTP-binding protein Rhoh</td>
<td>Negative regulator of hematopoietic progenitor cell proliferation, survival and migration</td>
</tr>
<tr>
<td>CYFIP2 (5q33.3)</td>
<td>0.0109 (0.9989)</td>
<td>cytoplasmic FMR1-interacting protein 2</td>
<td>Stimulates a migratory response in CD4+ lymphocytes, monocytes, and eosinophils</td>
</tr>
<tr>
<td>IL16 (15q26.3)</td>
<td>0.0128 (0.9880)</td>
<td>interleukin-16</td>
<td>Primes CD4+ T-cells for IL-2 and IL-15 responsiveness</td>
</tr>
<tr>
<td>IFI35 (17q21)</td>
<td>0.0184 (1.0007)</td>
<td>interferon-induced 35 kDa protein</td>
<td>Unknown</td>
</tr>
<tr>
<td>ENPP2 (8q24.1)</td>
<td>0.0192 (0.9996)</td>
<td>Ectonucleotide pyrophosphatase family member 2 autotaxin</td>
<td>Involved in several motility-related processes</td>
</tr>
<tr>
<td>CXCR4 (2q21)</td>
<td>0.0247 (0.9997)</td>
<td>C-X-C chemokine receptor type 4 stromal cell-derived factor 1 receptor</td>
<td>Receptor for CXCL12</td>
</tr>
<tr>
<td>BMP7 (20q13)</td>
<td>0.0292 (1.0061)</td>
<td>bone morphogenic protein 7</td>
<td>Induces cartilage and bone formation</td>
</tr>
<tr>
<td>CCR7 (17q12-21.2)</td>
<td>0.0311 (0.9986)</td>
<td>C-C chemokine receptor type 7</td>
<td>Receptor for MIP-3β (macrophage inflammatory protein 3 beta)</td>
</tr>
<tr>
<td>MS4A1 (11q12)</td>
<td>0.0351 (0.9993)</td>
<td>B-lymphocyte antigen CD20</td>
<td>Involved in the regulation of B-cell activation and proliferation</td>
</tr>
<tr>
<td>CCR6 (6q27)</td>
<td>0.0405 (0.9907)</td>
<td>C-C chemokine receptor type 6</td>
<td>Receptor for MIP-3α (macrophage inflammatory protein 3 alpha)</td>
</tr>
<tr>
<td>CCL19 (9p13)</td>
<td>0.0432 (0.9996)</td>
<td>CCL19 protein</td>
<td>Plays a role in inflammatory and immunological responses and also in normal lymphocyte recirculation and homing</td>
</tr>
<tr>
<td>CCL8 (17q11.2)</td>
<td>0.0446 (1.0009)</td>
<td>C-C motif chemokine 8 monocyte chemotactic protein 2</td>
<td>Chemotactic factor that attracts monocytes, lymphocytes, basophils and eosinophils</td>
</tr>
<tr>
<td>CCR2 (3p21.31)</td>
<td>0.0446 (0.9975)</td>
<td>C-C chemokine receptor type 2 monocyte chemotactrant protein 1 receptor</td>
<td>Receptor for the MCP (monocyte chemotactic protein)-1, 3 and 4</td>
</tr>
<tr>
<td>BST2 (19p13.1)</td>
<td>0.0468 (1.0002)</td>
<td>bone marrow stromal antigen 2 tetherin; CD317</td>
<td>Involved in pre-B-cell growth</td>
</tr>
</tbody>
</table>
References shown in [ ]
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Kei Suzuki, Stefan S Kachala, Kyuichi Kadota, et al.

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