

# High expression of CD20<sup>+</sup> lymphocytes in soft tissue sarcomas is a positive prognostic indicator

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The immune status is important in cancer patients. Tissue microarrays from 249 patients with soft tissue sarcomas were constructed. Immunohistochemistry was used to evaluate the CD3<sup>+</sup>, CD4<sup>+</sup>, CD8<sup>+</sup>, CD20<sup>+</sup> and CD45<sup>+</sup> lymphocytes in tumors. High density of CD20<sup>+</sup> lymphocytes is an independent positive prognostic indicator for these patients.

## Introduction

Soft tissue sarcomas (STS) are relatively rare, heterogeneous malignancies of mesenchymal origin with a high mortality rate. They comprise less than 1% of adult malignancies and approximately 50% of the STS patients will succumb to their disease because of metastasis or local relapse. There are several prognostic factors which determine tumor progression, and ultimately the patient's outcome, including positive resection margins; presence of local recurrence; and tumor grade, size, location, depth and histological entity. The purpose of this study<sup>1</sup> was to clarify the prognostic significance of lymphocyte infiltration in non-gastrointestinal stromal tumor (GIST) STSs.

## Results

**Clinicopathological variables.** Patient age range was 0–91 years (mean 55 years), and 44% of the patients were males. The non-GIST STS comprised 68 undifferentiated pleomorphic sarcoma, 67 leiomyosarcoma, 34 liposarcoma, 20 malignant fibroblastic/myofibroblastic tumors, 16 rhabdomyosarcoma, 16 synovial sarcoma, 13 angiosarcoma, 11 malignant peripheral nerve sheath tumors (MPNST) and 4 other STS. There were 61 low grade STS

(24%) and 188 high grade (FNCLCC grade 2 and 3) STS (76%).

The treatment option of choice was surgery (n = 228): 118 patients received surgery only; 55 patients received surgery and radiotherapy; 40 patients received surgery and chemotherapy; 13 patients received surgery, radiotherapy and chemotherapy; 2 patients received chemotherapy only; 3 patients received chemotherapy and radiotherapy; 2 patients received radiotherapy only; and 16 patients received no therapy. The 5-year survival with non-wide resection margins was 33% and with wide resection margins it was 62%.

**Univariate analyses.** Nationality, tumor size, malignancy grade, tumor depth, metastasis at time of diagnosis, surgery and surgical margins were all significant indicators for disease-specific survival (DSS) in univariate analyses. Most of the patients with non-GIST STS who did not survive their disease, died within the first 10 years (120 months). After 10 years almost 60% (n = 108) of the patients with wide resection margins were alive, but only 20% (n = 141) of patients with non-wide resection margins or no surgery (p < 0.001).

Furthermore, increasing numbers of CD4<sup>+</sup> (p = 0.008) and CD20<sup>+</sup> lymphocytes in tumor (p = 0.006) correlated significantly with an improved DSS in patients

with wide resection margins (n = 108) **Figure 1.** In patients with non-wide resection margins (n = 141) increasing numbers of CD3<sup>+</sup> lymphocytes correlated significantly (p = 0.028) with shorter DSS.

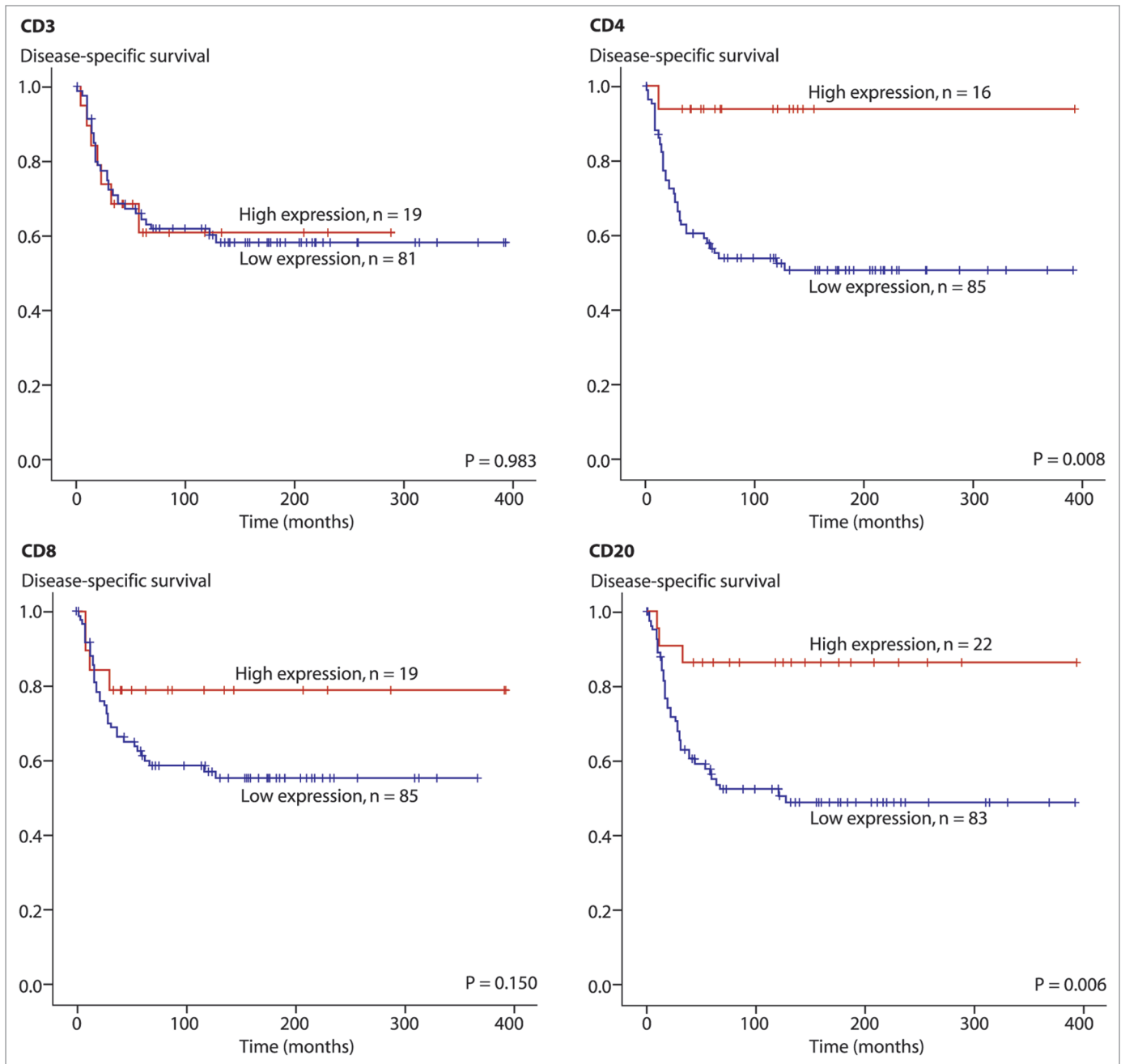
**Multivariate analyses.** An independent positive prognostic factor for improved DSS in patients with wide resection margin was a high number of CD20<sup>+</sup> lymphocytes in the tumor (HR 5.5, CI 95% 1.62–18.61, p = 0.006).

## Discussion

Activation of the adaptive immune system may suppress malignant cells, whereas activation of various types of innate immune cells may promote tumor growth.<sup>3</sup> The adaptive immunity, orchestrated by antigen-specific T and B-lymphocytes, inhibits tumor growth through both direct killing by cytotoxic T-lymphocytes, and a combination of cytokine and antibody mediated tumor cell lysis.<sup>3</sup> Cancer infiltration by tumor reactive T-lymphocytes is required for efficient tumor eradication.<sup>4</sup> However, cancer cells can escape the immune system in several ways including suppression of cytotoxic T-cells, by regulatory T-cells and by accumulation of myeloid suppressor cells.<sup>4</sup>

The role of CD4<sup>+</sup> T and B lymphocytes is controversial in many cancers

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**Figure 1.** Disease-specific survival curves for CD3<sup>+</sup>, CD4<sup>+</sup>, CD8<sup>+</sup>, CD20<sup>+</sup> and CD45<sup>+</sup> lymphocytes in STS with wide resection margins.

including STS; CD4<sup>+</sup> cells in the absence of the CD8<sup>+</sup> cytotoxic T cells are critical and sufficient for NKT cell-dependent rejection of experimental tumors.<sup>5</sup> In lung cancer the prognostic impact of CD4 is controversial,<sup>6</sup> but in our material CD4<sup>+</sup> cells were a positive prognostic factor in univariate analyses.

CD20<sup>+</sup> cells are associated with a better survival in lung cancer, cervical cancer, prostate cancer and ovarian cancer.<sup>6</sup>

CD20<sup>+</sup> B-cells in metastatic lymph nodes are associated with favorable outcome in patients with oro- and hypopharyngeal carcinoma.<sup>7</sup> On the other hand, B-cell infiltration detected by flowcytometry with CD19 were correlated with unfavorable outcome in metastatic ovarian carcinoma.<sup>8</sup> In our material high density of CD20<sup>+</sup> lymphocytes was an independent positive prognostic indicator. This may suggest that CD20<sup>+</sup> cells in the tumor are

mediating a strong anti-tumor immune response in STS, but this effect is not strong enough to improve survival in patients without wide resection margins.

Among STS patients who have had wide resection margins, it will be essential to identify those who will relapse and succumb this disease as these patients may benefit from adjuvant therapy, including immunotherapy. Until now adjuvant chemotherapy has been

controversial due to inadequate selection criteria.

High density of CD20<sup>+</sup> lymphocytes in STS with wide resection margins is an independent positive prognostic indicator. Further research to define if CD20<sup>+</sup> cells can modify tumors in a way that reduces disease progression and metastatic potential is needed.

## Materials and Methods

**Patients and clinical samples.** Primary tumor tissues from patients diagnosed with STS at the University Hospital of North Norway (UNN) from 1973 to 2006 and the Hospitals of Arkhangelsk region, Russia, were used in this retrospective study. 249 patients were eligible for this study with complete medical records and adequate paraffin-embedded

tissues blocks. The median follow-up was 38 (range 0–392) months.

Tissue microarrays (TMAs) were constructed for high-throughput molecular pathology research.<sup>2</sup> The ARIOL imaging system (Genetix, San Jose, CA) was used to scan the slides for antibody staining of the tissue micro arrays (TMAs).

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