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# Oncostatin M and Nivolumab Affect the Cytotoxic T-Cell Proportions and the Susceptibility to TRAIL-Induced Death in Non-Leukocyte Cell Subpopulations in Soft Tissue Sarcomas

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**Keywords**

Soft tissue sarcomas · Oncostatin M · Nivolumab · T cells · Cytokines · Monoclonal antibody · Tumor microenvironment

**Abstract**

***Introduction:*** Soft tissue sarcomas (STSs) are malignant tumors arising from mesenchymal tissues. Patients with advanced and metastatic STSs have low overall survival rates and relatively limited treatment options. Oncostatin M (OSM) is a pleiotropic cytokine that was shown to carry both pro- and anti-tumorigenic properties in various cancer types. However, the role of OSM in STSs has not yet been elucidated. Moreover, the potential additive effects of combining OSM and anti-PD-1 therapy have not been carried out so far. ***Methods:*** The aim of this study was to determine the effects of in vitro OSM administration on liposarcoma, leiomyosarcoma, and myxofibrosarcoma immune cells isolated from peripheral blood and tumor tissues and the potential cooperative nature of OSM and nivolumab in treating these STSs. We designed a cohort study to explore novel histology-driven therapies in our target STSs. The immune cells were isolated from the peripheral blood and tumors of patients with STS, and the proportions and phenotypes of immune cells were evaluated with flow cytometry after cultivation with therapeutic monoclonal antibodies. ***Results:*** The proportion of peripheral $CD45^+$ cells was not affected by OSM but was significantly increased by nivolumab, whereas both treatments had an effect on $CD8^+$ T cells. In tumor tissues, $CD8^+$ T cell and $CD45^-$ $TRAIL^+$ cell cultures were boosted by nivolumab and significantly enriched by OSM. Our data suggest that OSM may play a role in the treatment of leiomyosarcoma, myxofibrosarcoma, and liposarcoma. ***Conclusion:*** In conclusion, the biological

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efficacy of OSM is reflected in the tumor microenvironment rather than in the peripheral blood of the patients in our cohort, and nivolumab could potentiate its mechanism of action in selected cases. Nevertheless, more histotype-tailored studies are needed to fully understand the functions of OSM in STSs.



## Introduction

Soft tissue sarcomas (STSs) are rare malignancies that originate in the mesenchymal tissues [1]. Liposarcoma, leiomyosarcoma, and myxofibrosarcoma are the most prevalent STS subtypes; however, over 100 distinct histological and molecular subtypes have been identified to date [2, 3]. Given the differences in tumor biology, patterns of recurrence/progression and varying sensitivities to systemic treatments, histotype, and patient characteristics must be considered when deciding whether or not to apply specific types of therapy [2]. Surgical resection is the first-line treatment for resectable tumors [3]. However, approximately 50% of patients with high-grade STSs die of metastatic disease [4]. Systemic treatments for advanced and/or metastatic STSs rarely improve overall survival [5]. The advent of novel therapeutic agents has altered the treatment landscape of metastatic diseases and has proven to be of clinical value [6, 7]. These therapeutic agents include immunotherapies with immune checkpoint inhibitors, cytokines, and monoclonal antibodies [8–10].

Oncostatin M (OSM) is a pleiotropic cytokine belonging to the IL-6 cytokine family [11]. The human OSM gene is located on chromosome 22ql2 with the final OSM protein containing 252 amino acids [12]. The posttranslational modifications of OSM are cell-type dependent, and activated immune cells, such as T cells, macrophages, or dendritic cells, are the main source of OSM [12]. The OSM gene is adjacent to the gene for leukemia inhibitory factor (LIF), and thus, OSM shares a homology with LIF in both function and structure [13]. Two separate receptor complexes, heterodimers gp130/LIFRβ and heterodimer gp130/OSMRβ, are responsible for OSM biological effects [12]. Unfortunately, the OSM cytokine/receptor interactions are poorly understood, and data from human studies are currently very limited [13]. In addition, it has been shown that human and murine OSM receptors are quite heterogenous [14].

Although OSM has been shown to play a crucial role in regulating cross-talk between immune and cancer cells and cancer-associated fibroblasts, its mechanism of action in the tumor microenvironment (TME) remains unknown [13]. Together with IL-6, OSM plays a vital role in the modulation of inflammatory responses [11]. Although OSM was originally described as an antitumor cytokine, recent studies have highlighted its role in tumor progression in selected cancer types [13]. These protumoral effects have been primarily attributed to the overexpression of OSM in brain tumors, prostate cancer, and several lymphoproliferative malignancies, as well as to the broad involvement of OSM in cell proliferation and angiogenesis [15–18]. In breast cancer, OSM induces vascular endothelial growth factor and thus promotes the invasive capacity of tumor cells [19]. Similar observations have been reported for osteosarcoma [20]. In contrast, high levels of OSM in the chondrosarcoma TME were shown to significantly affect apoptosis of tumor cells, thus limiting tumor proliferation [21]. These conflicting biological mechanisms caused by OSM imply that diverse signaling pathways trigger pro- or anti-tumorigenic events in the TME [13]. In addition, OSM also regulates extracellular matrix remodeling, drug resistance, metastatic capacity, and inflammation [22]. All these aspects presumably also control the pro- and anti-tumorigenic events in cancers. Several anti-tumoral functions of OSM were shown to act through the MEK/ERK signaling pathway, resulting in the inhibition of cell growth [23]. Other studies demonstrated the ability of OSM to increase mRNA levels and protein stability by enhancing the expression of CDK inhibitors [24]. The biological effects promoting tumorigenesis were described by studies which indicated that OSM promotes proliferation through OSMR and STAT3 signaling [25, 26]. Accordingly, OSMR was shown to be overexpressed in several cancers, such as advanced squamous cell carcinoma, pancreatic ductal adenocarcinoma, and gastric tumors [27]. Genetic analyses further demonstrated that OSMR regulates the local immune response and could serve as a prognostic factor in patients with glioblastoma [17]. Nivolumab, an anti-PD1 monoclonal antibody, has already been approved as a first-line treatment for adult patients with several cancer types. Currently, there are ongoing clinical trials in patients with STSs to evaluate the efficacy of nivolumab as either monotherapy or combination therapy [1]. Nivolumab and OSM interact with specific receptors and trigger a cascade of reactions, as shown in Figure 1.

Despite advances in the treatment of advanced/metastatic STSs, outcomes remain poor and treatment strategies are limited [1, 3, 28]. This is predominantly due to the rarity and heterogeneity among STSs, which

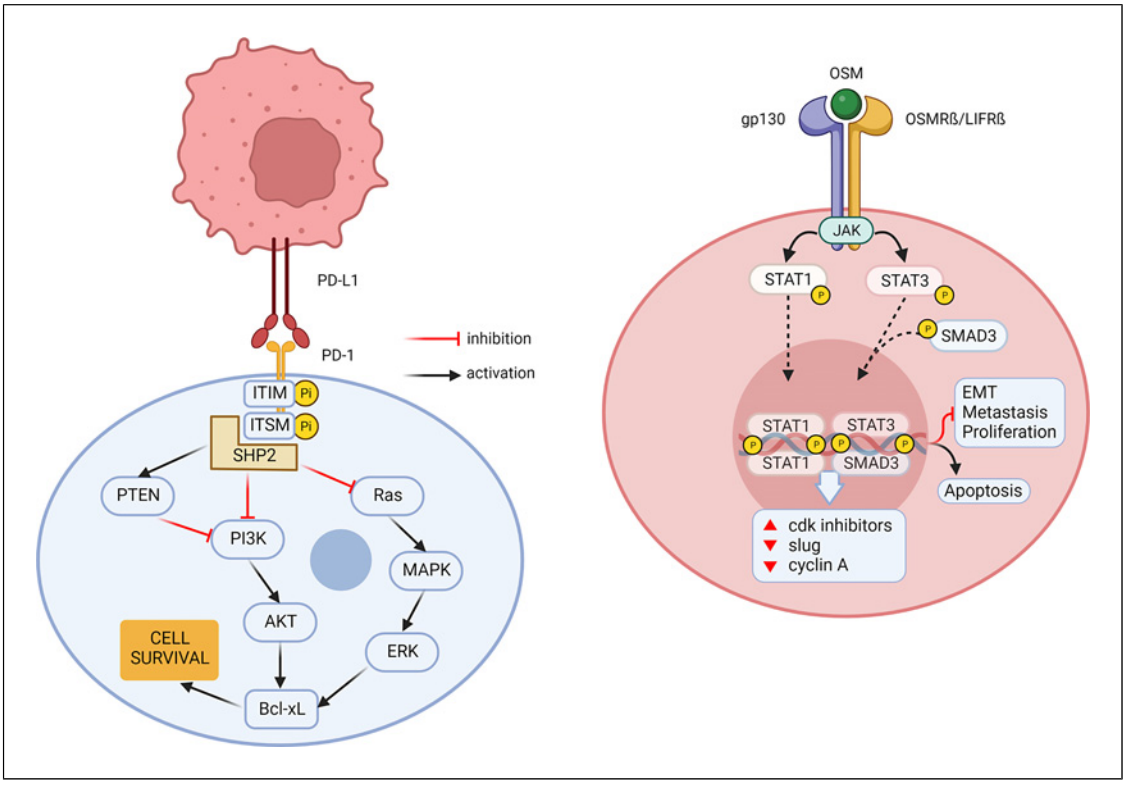


**Fig. 1.** PD-1 and oncostatin M (OSM) signaling pathway. Left section: Binding of PD-1 to its ligand PD-L1 leads to a phosphorylation of the ITIMs and ITSMs which are present in the intracellular domain of the PD-1 receptor. Upon this binding, tyrosine acid phosphatase SHP-2 is recruited and further suppresses the PI3K/AKT signaling pathway and Ras/MAPK/ERK signaling pathway. Right section: OSM binds first to gp130 with low affinity and then recruits either LIFR or OSMR. All these components of the OSM receptor associate with the JAK family of tyrosine kinases through their cytoplasmic domains. This leads further to the phosphorylation, dimerization, and nuclear translocation of STAT family proteins. STATs are then tyrosine-phosphorylated and translocated to the nucleus, where they control transcription of diverse target genes. Created with BioRender software (Agreement No. RF24FVICAZ). AKT, protein kinase B; ERK, extracellular signal-regulated kinase; ITIM, immunoreceptor tyrosine-based inhibitory motif; ITSM, immunoreceptor tyrosine-based switch motif; JAK, Janus family kinases; LIFR, leukemia inhibitory factor receptor; MAPK, mitogen-activated protein kinase; OSM, oncostatin M; OSMR, oncostatin M receptor; PD-1, programmed cell death 1; PD-L1, programed cell death ligand 1; PI3K, phosphatidylinositol 3-kinase; SHP-2, SH2 domain-containing protein tyrosine phosphatase-2; STAT, signal transducer and activator of transcription.

hamper the design of robust clinical trials with histology-specific or widely standardized approaches [1]. To date, studies have identified STS subtypes that are more likely to benefit from the treatment with immunotherapy. Among those subtypes, liposarcoma and leiomyosarcoma were shown to have the highest expression of immune checkpoint molecules [29]. Moreover, patients with liposarcoma were shown in clinical trials to respond well to anti-PD-1 immunotherapy [30]. Another study in STSs patients demonstrated a superior efficacy of anti-PD-1 combinatorial administration as compared to anti-PD-1 monotherapy [32]. The effects of OSM on STS tumors have not yet been evaluated, although a single in vitro study has demonstrated a direct anti-cancer effect of OSM on chondrosarcoma [21].

Therefore, we aimed to clarify the effects of in vitro OSM administration on immune cells isolated from liposarcoma, leiomyosarcoma, and myxofibrosarcoma. We also investigated the potential cooperative nature of OSM and nivolumab. To develop histology-driven therapies, we created a cohort of patients with liposarcomas, leiomyosarcomas, and myxofibrosarcomas and analyzed the effects of OSM administration on the immune cells isolated from peripheral blood and tumor tissues of the participants and observed changes in cell proportions and phenotypes. We also evaluated the co-administration of nivolumab and OSM to explore combination regimens in STSs.

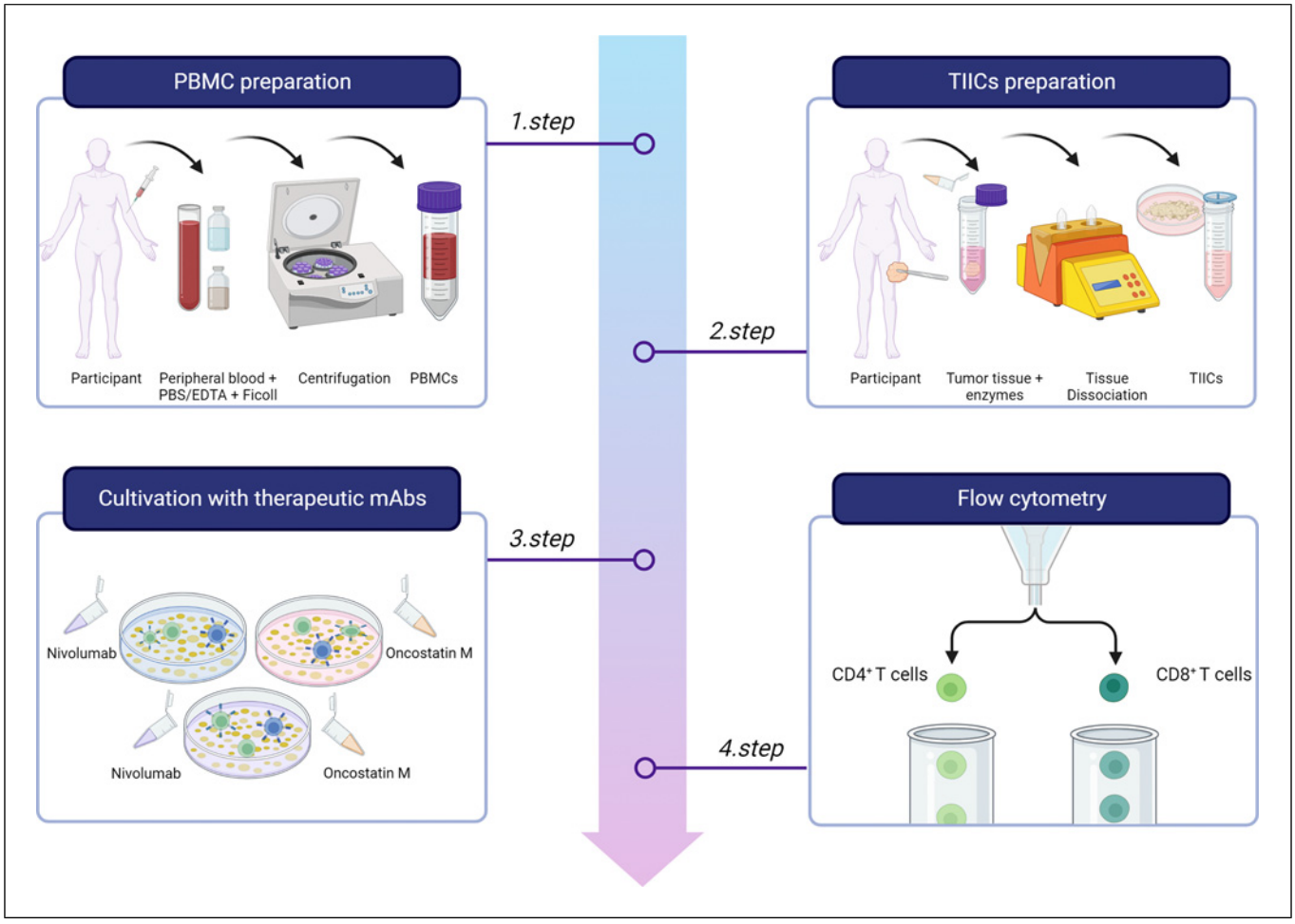


**Fig. 2.** Experimental design of the study. Step 1: To obtain purified peripheral blood mononuclear cells (PBMCs) from all study participants; 9 mL of peripheral blood was collected from each participant in an EDTA-blood collection tube. The Ficoll-Paque separation technique with density gradient centrifugation was used to distribute cells based on the differences in their density/size. Step 2: Tumor tissue was obtained from two study participants. The tumor was mechanically and enzymatically dissociated, and cell suspensions with tumor-infiltrating immune cells (TIICs) were obtained. Step 3: Cell suspensions isolated from peripheral blood and tumors of STS patients were incubated with either nivolumab alone, OSM alone, or a combination of nivolumab and OSM. These conditions were also accompanied by a costimulation with anti-CD3/CD28 Dynabeads. Step 4: Flow cytometry was used to evaluate the proportions and phenotypes of the immune cells. Created with BioRender.com (Agreement No. UG24FVIP5C).

## Materials and Methods

*Study Cohort*

Fifteen participants were enrolled in the study between January and September 2022. The female-to-male ratio was 8:7. The mean age was 63.5 and ranged from 38 to 83 years. Peripheral blood mononuclear cells (PBMCs) and tumor-infiltrating immune cells (TIICs) were analyzed in ten and two patients, respectively. The following histological subtypes of STS were selected: well-differentiated liposarcoma, myxofibrosarcoma, and leiomyosarcoma. The control group included three healthy donors. The sequential experimental design is shown in Figure 2.

The ethical principles for medical research involving humans strictly adhered to the World Medical Association Declaration of Helsinki. All participants provided written consent to participate in the study, which was approved by the Ethics Committee for Multi-Centric Clinical Trials of the University Hospital Motol (reference No.: EK-189/20).

*Preparation of PBMCs and TIICs*

Peripheral blood (≥9 mL per patient) was collected from each participant in an EDTA-blood collection tube. A recommended standard method for cell separation and mononuclear cell isolation using Ficoll-Paque was followed [32]. We used density gradient centrifugation (30 min, RT, 1,800 rpm) to distribute cells in the solution in layers based on the differences in their density/size. Purified PBMCs from all study participants (patients with STSs and healthy donors) were obtained.

TIICs were isolated from surgical resections, and tissue samples were placed on a sterile eight-well desk. Initially, each sample was mechanically dissociated using a gentleMACS™ Dissociator (MACS Miltenyi Biotec; gentleMACs Program h_tumor_01) [33, 34].

The dissociated tissue was then incubated with 5 mL of RPMI-1640 medium (Gibco™ RPMI 1640 Medium, Thermo Fisher Scientific) at room temperature and enzymatically processed according to a previously published protocol [4, 34]. The cell suspension was filtered through a 100-μm cell strainer, and all debris/cell clumps were removed. The cell suspension was washed in phosphate-buffered saline (PBS), and red blood cells were removed with ammonium-chloride-potassium lysing buffer (Thermo Fisher Scientific). After centrifugation (500× g, 10 min, RT), the cell pellets were resuspended in cold PBS (4°C) with 2 mM EDTA (PBS/EDTA). Cells were placed in a cryopreservation medium at −80°C for 1 day and then stored in liquid nitrogen.

*Selective T-Cell Stimulation and Cell Cultures*

Cells obtained from the peripheral blood and tumor tissue samples were slowly defrosted, centrifuged, transferred into a 96-well flat-bottom plate, and supplemented with RPMI-1640 medium (Gibco™ RPMI 1640 Medium, Thermo Fisher Scientific). Non-stimulated cell suspension samples from all participants were defrosted and analyzed on the day of the flow cytometry analysis.

In the first therapeutic condition, the cell suspensions isolated from the peripheral blood and tumor tissue samples were activated by superparamagnetic polymer beads with an optimized mixture of monoclonal antibodies against CD3 and CD28 surface molecules (Dynabeads Human T-Activator CD3/CD28, Gibco, Thermo Fisher Scientific) and stimulated for 20 h.

To evaluate the effect of OSM on the immune cells isolated from the peripheral blood and tumor tissues, cell suspensions were also stimulated with anti-CD3/CD28 Dynabeads and incubated for 20 h with OSM cytokines (Bio-Techne, R&D Systems) at a final concentration of 2 μg/mL. For the remaining therapeutic conditions, cell suspensions were also stimulated by anti-CD3/CD28 Dynabeads and cultured for 20 h with anti-PD1 monoclonal antibody (mAb, Nivolumab, Selleckchem, Germany) at a final concentration of 2 μg/mL. In the fifth study condition, the cell suspensions were cultured with anti-CD3/CD28 Dynabeads, OSM, and nivolumab.

*Flow Cytometry*

The cultured cells were stained with fluorophore-conjugated protein-specific antibodies. The following monoclonal antibodies (mAbs) were used: anti-CD45, CD3, CD4, CD8, and TRAIL-R (all BioLegend). After a 30-min incubation, the isolated cells stained with mAbs were washed and analyzed using a BD LSRFortessa flow cytometer (Becton Dickinson).

*Data Analysis*

Flow cytometry data were analyzed using FlowJo software (version 10.6.1). Basic descriptive statistics were calculated separately for the three groups of samples (PBMCs, TIICs, and healthy donors), which were compared using one-way ANOVA with least significant difference post hoc tests. Differences between therapeutic conditions (non-stimulated, stimulated with anti-CD3/CD28 Dynabeads, co-cultured with OSM, co-cultured with nivolumab, and co-cultured with OSM + nivolumab) were evaluated using two-way repeated ANOVA with one between-sample group (PBMCs, TIICs, and healthy donors), one within-sample group (therapeutic condition) factor, and an interaction term between factors (sample group-therapeutic condition). Since the interaction effect was statistically significant in most tested variables (indicating that the differences between therapeutic conditions were not constant among the tested groups), one-way repeated ANOVA followed by least significant difference post hoc tests were used separately in each group of patients as the next step. Bivariate associations between the variables were assessed using the Pearson product-moment correlation coefficient. Differences were considered statistically significant at a two-tailed $p < 0.05$. Statistical analyses and results visualization were performed using IBM SPSS (version 25; IBM SPSS, Armonk, NY, USA) and ggplot2 (Wickham, 2016) packages in R software (R Foundation, Vienna, Austria).

## Results

*Nivolumab Increases the Proportions of CD45$^+$ TIICs but Does Not Affect Peripheral Leukocytes*

We investigated the effects of OSM, nivolumab, and their combinatorial administration on leukocytes isolated from the peripheral blood of patients with STS. Generally, most circulating PBMCs are naive or resting cells with low effector functions; therefore, we determined whether adding OSM and/or nivolumab could enhance naive leukocyte proliferation and activation. The peripheral blood of patients with STS after erythrocyte lysis contained a substantially high proportion of CD45$^+$ cells (approximately 95%). Adding costimulatory molecules and therapeutic agents for 20 h did not increase the proportion of CD45$^+$ cells (Fig. 3a).

However, the treatment appeared to affect the proportion of T cells as we observed an almost 30% decrease in CD3$^+$ cells under all costimulatory conditions (costim. alone, costim. + nivolumab, costim. + OSM, and costim. + OSM + nivo). Stimulated T cells are known to downregulate TCR and CD3 after activation. However, data indicated that nivolumab and OSM addition did not enhance PBMC T-cell proliferation or activation [35].

Notably, when extending the analyses to the tumor compartment, we observed a rapid increase in the proportion of CD45$^+$ cells after stimulation with costimulatory molecules. This was particularly relevant since CD45 is considered a pan-leukocyte marker and its high expression generally correlates with the TIL infiltration [36]. Moreover, high levels of CD45 were shown to significantly increase the local recurrence-free survival and the disease-specific survival in patients with head and neck cancer [36]. Similarly, the expression level of CD45 was related to good prognosis in multiple tumors, such as lung adenocarcinoma or skin cutaneous melanoma [37].

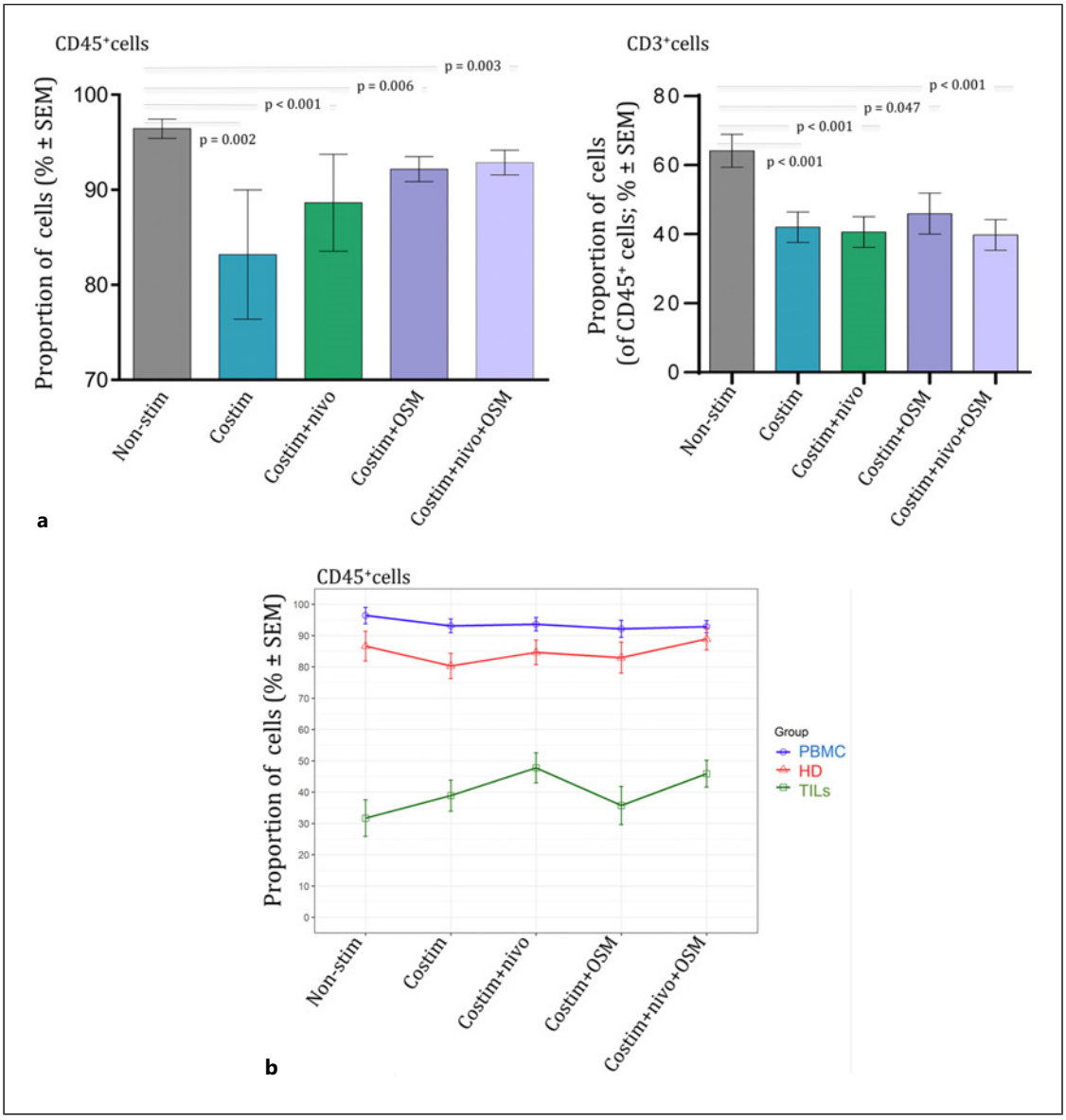


**Fig. 3.** Differential responses to stimulation of peripheral and TIICs. **a** $CD45^+$ cells and $CD3^+$ cells isolated from the peripheral blood of the study participants decreased after 20 h of stimulation with anti-CD3/CD28 Dynabeads (costim.); with anti-CD3/CD28 Dynabeads and nivolumab; with CD3/CD28 Dynabeads and OSM; and with CD3/CD28 Dynabeads, nivolumab, and OSM. **b** Rapid increase in the proportions of $CD45^+$ TIICs was observed after stimulation with anti-CD3/CD28 Dynabeads and nivolumab.

Interestingly, the phenomenon was even more profound after nivolumab administration (Fig. 3b). However, the addition of OSM did not seem to enhance the expansion of the $CD45^+$ subpopulation in either the costimulation or the costimulation + nivolumab treatment groups. Furthermore, the proportion of $CD3^+$ TIICs did not exhibit any significant changes after nivolumab/OSM administration in vitro (data not shown).

### *Nivolumab and OSM Administration in vitro Alters the Proportion of Peripheral $CD8^+$ T Cells from Patients with Soft Tissue Sarcomas*

Our analysis indicates that the proportion of $CD45^+$ cells in TIICs can be increased by the administration of nivolumab combined with CD3/CD28 stimulation but not by the addition of OSM. Despite the treatments have being aimed at activating T cells, we did not detect any

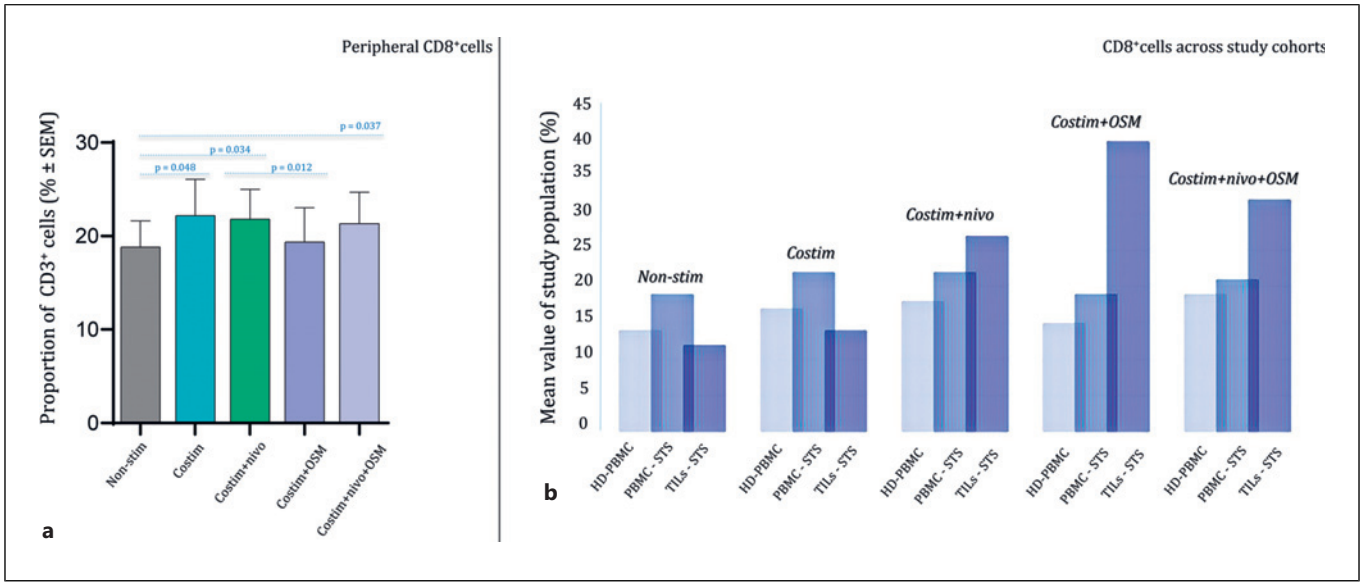


**Fig. 4.** Changes in the proportions of CD8$^+$ T cells. **a** Graph showing a significant enrichment of the peripheral CD8$^+$ T cells after the addition of CD3/CD28 Dynabeads and after the addition of CD3/CD28 Dynabeads + nivolumab. **b** Differential responses of CD8$^+$ T cells to five therapeutic conditions (non-stimulated cell culture, cell culture stimulated with CD3/CD28 Dynabeads, cell culture supplemented with CD3/CD28 Dynabeads and nivolumab, cell culture supplemented with CD3/CD28 Dynabeads and OSM, cell culture supplemented with CD3/CD28 Dynabeads + nivolumab + OSM) based on the source of the cells (peripheral blood of healthy donors, peripheral blood of sarcoma patients, tumor-infiltrating lymphocytes isolated from tumors of sarcoma patients). HD, healthy donor; PBMC, peripheral blood mononuclear cells; STS soft tissue sarcoma; TILs, tumor-infiltrating lymphocytes.

significant changes in the proportion of CD3$^+$ TIIC T cells after nivolumab/OSM administration in vitro.

We hypothesized that the treatments might affect the proportion of a particular T-cell subset, and this change is lost when looking at CD3$^+$ cells as a whole. Therefore, we investigated whether the proportions of CD4$^+$ and CD8$^+$ T cells could be affected by OSM or nivolumab.

While peripheral CD4$^+$ T cells displayed similar tendencies as peripheral CD45$^+$ cells (data not shown), we observed significant changes in the CD8$^+$ T-cell subpopulation. Peripheral CD8$^+$ T cells were significantly enriched after the addition of CD3/CD28 Dynabeads ($p$ = 0.048) and after the addition of nivolumab ($p$ = 0.034) (Fig. 4a). Supplementation of nivolumab-treated cell culture with OSM slightly increased the proportion of CD8$^+$ T cells (Fig. 4a). Even though the number of our study participants was not sufficient to compare differences among the three histological subtypes, we have evaluated the proportions of peripheral CD8$^+$ T cells as the sum of all values for each histological subtype to investigate whether well-differentiated liposarcoma, myxofibrosarcoma, or leiomyosarcoma could display dissimilar responses based on the in vitro condition (non-stim, CD3/CD28 Dynabeads, nivolumab, OSM, CD3/CD28 Dynabeads + nivolumab + OSM). As anticipated, there were no contrasting responses among the histological subtypes of STSs in our study cohort. Oppositely, we noticed varying responses to therapeutic agents across study populations which were most evident in the cells isolated from the tumors of patients with STS (Fig. 4b).

The expression of immune checkpoint molecules has been extensively investigated in large multicentric studies with PD-1 and PD-L1 expression being observed in only 10 and 22% of sarcoma cases, respectively [29]. Given the limited number of our study participants and the primary focus on the immune cell activation rather than the exhaustion, we have only analyzed the expression of PD-L1 in CD8$^+$ T cells. We also aimed to analyze the PD-L1$^+$ CD8$^+$ T cells because of their previous association with progression-free survival in non-small-cell lung carcinoma patients treated with immune checkpoint inhibitors [38]. Even though the proportions of PD-L1$^+$ CD8$^+$ T cells were relatively high in all patients, we did not observe any significant differences in PD-L1 expression with the addition of CD3/CD28 Dynabeads or with the addition of nivolumab or OSM (online suppl. Fig. 1; for all online suppl. material, see www.karger.com/doi/10.1159/000529811).

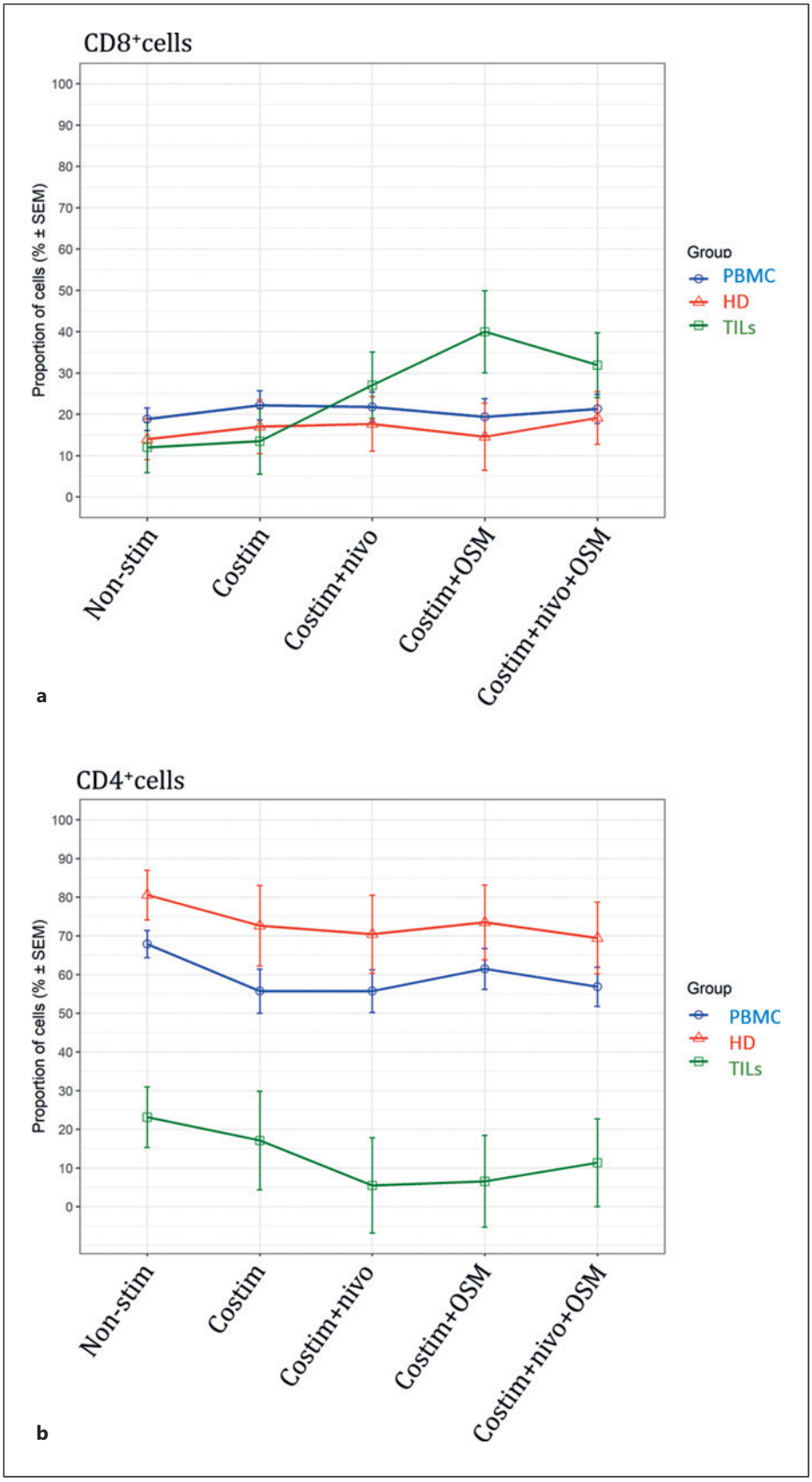


**Fig. 5.** $CD8^+$ T cells isolated from the tumor tissues are affected by OSM. **a** Rapid increase in the proportion of $CD8^+$ T cells after nivolumab and OSM administration. **b** OSM did not increase the proportions of $CD4^+$ T cells isolated from the tumor tissues.

*OSM Causes a Significant Increase in the Proportion of CD8$^+$ TIICs*

The observations in CD8$^+$ T cells prompted us to further investigate this subpopulation after isolation from the tumor compartment of patients with STS. We also correlated the findings from Section 3.2 with healthy donors and observed no significant differences compared to the peripheral CD8$^+$ T cells of patients with STS. However, in TIICs, we observed a rapid increase in the proportion of CD8$^+$ T cells after nivolumab administration and an even greater increase after OSM administration (Fig. 5a). The combinatorial administration of nivolumab and OSM did not appear to potentiate the efficacy of either treatment. In CD4$^+$ T cells isolated from the tumoral compartment, administration of nivolumab and OSM did not enhance the proportion of the subpopulation (Fig. 5b).

*Nivolumab and OSM Increase the Susceptibility to Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand-Induced Death in Non-Leukocyte Cells in the TME of Patients with Sarcoma*

So far, data indicated that nivolumab and OSM have an effect on CD8$^+$ T cells of patients with STSs. While nivolumab mainly promoted the enrichment of peripheral CD8$^+$ T cells, OSM had a significant effect on CD8$^+$ TIICs.

In our previous studies, we demonstrated that the expression of death receptors might reflect the susceptibility of a particular cell subset to ligand-induced cell death in the TME [39]. We also highlighted the importance of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL; CD253) and the TRAIL receptor signaling pathway [40]. The binding of TRAIL to TRAIL-receptor selectively triggers the extrinsic apoptotic pathway in malignant cells [40]. Therefore, we analyzed the expression of TRAIL receptors in the CD45$^-$ cell subpopulation to evaluate whether OSM or nivolumab impacted the apoptosis of non-leukocyte cell subpopulations of the TME (this subpopulation also includes tumor cells). Indeed, the observed change in the proportion of CD45$^+$ cells might result from enhanced apoptosis in the CD45$^-$ compartment.

The non-leukocyte cell subpopulations in the peripheral blood of patients with STS and healthy donors did not exhibit any significant changes after costimulation or OSM and nivolumab administration. However, in the tumoral compartment, we observed a decrease in the proportion of TME-infiltrating non-leukocyte cells after costimulation and nivolumab administration (Fig. 6a). While OSM did not significantly decrease the CD45$^-$ cell subpopulation, it caused an increase in CD45$^-$ TRAIL$^+$ cells in the TME (Fig. 6b). The addition of nivolumab to OSM did not further increase the number of CD45$^-$ TRAIL$^+$ cells, although nivolumab alone displayed excellent efficacy. In peripheral non-leukocyte cells, which may contain circulating tumor cells, nivolumab appeared to have similar (augmenting) effects on the proportions of CD45$^-$ TRAIL$^+$ cells, whereas OSM did not (Fig. 6c).

## Discussion

The rarity and heterogeneity among STSs hamper the design of robust clinical trials with histology-specific or widely standardized approaches [1]. Therefore, we selected patients with liposarcoma, leiomyosarcoma, or myxofibrosarcoma to develop therapeutic strategies tailored to a specific histotype. In these patients, we investigated the effects of OSM with or without nivolumab on immune cells isolated from peripheral blood or tumor tissue. We also selected nivolumab, a well-known and widely used immune checkpoint inhibitor, to serve as a potential promoter of OSM function. Initially, we explored the peripheral leukocytes of study participants and observed that OSM did not affect the proportion of peripheral CD45$^+$ cells. However, nivolumab effectively increased CD45$^+$ cells. This phenomenon might be of clinical relevance since CD45 was previously associated with good prognosis in multiple tumors. However, larger histotype-tailored studies are needed to determine the relationship between CD45$^+$ cells and prognostic indicators, such as disease-free survival and overall survival, in patients with STSs.

When analyzing specific T-cell subpopulations, we observed the effects of both nivolumab and OSM on the proportion of CD8$^+$ T cells but not on the proportion of CD4$^+$ T cells. These results suggest that both agents predominantly act on CD8$^+$ T-cell subpopulations, although the expression of PD-L1 was neither affected by OSM nor by nivolumab. To decipher any relevant associations between STS histological subtype and response to immunotherapy, we have also correlated the responses between well-differentiated liposarcoma, myxofibrosarcoma, and leiomyosarcoma. Unfortunately, we did not observe a greater response to therapy in any of these subtypes. However, our cohort was rather small to draw these conclusions, and thus, it is crucial to evaluate these findings in larger cohorts.

When extending our analyses to the tumor tissue compartment, nivolumab also appeared to increase CD8$^+$

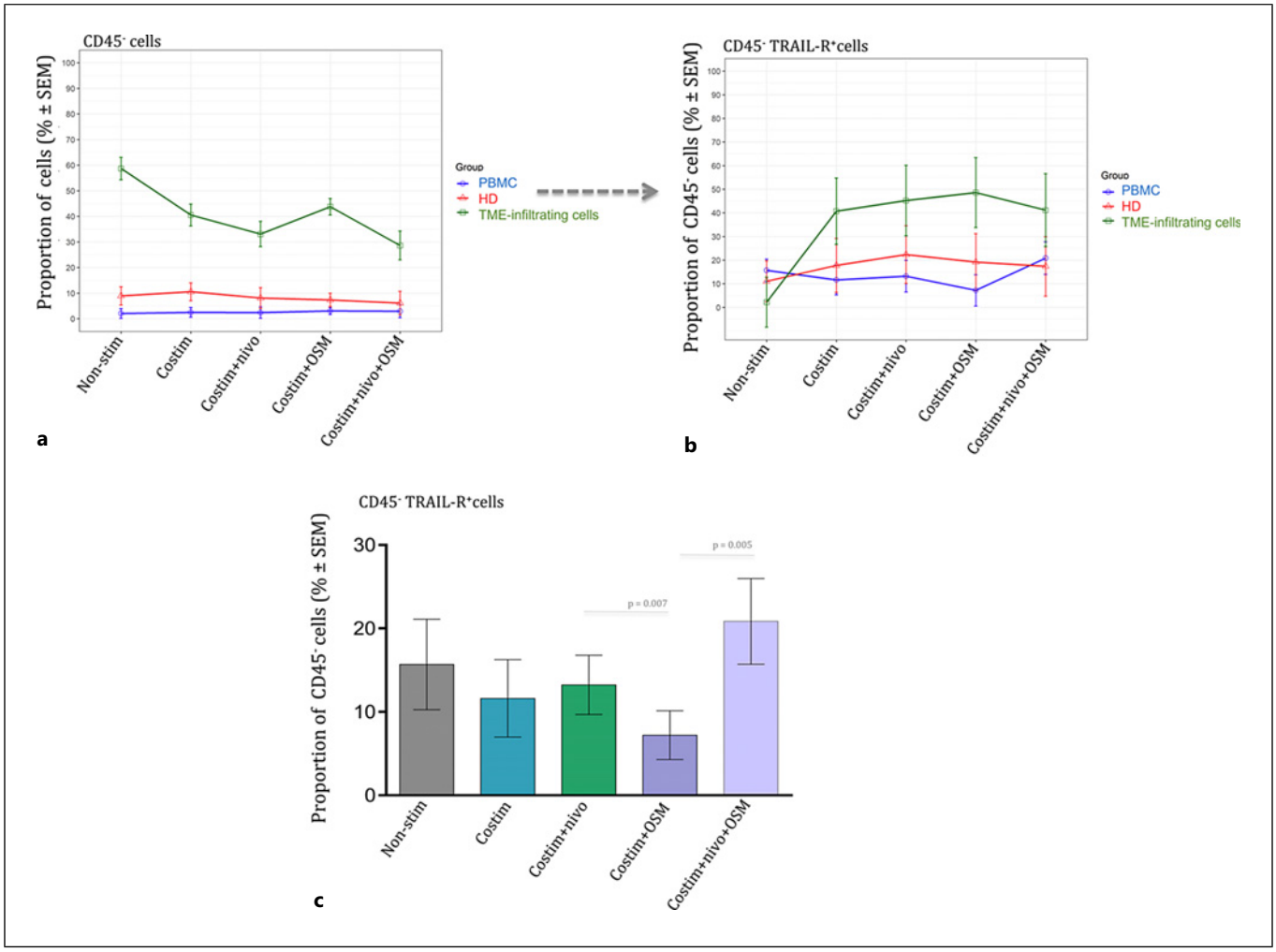


**Fig. 6.** Effects of nivolumab and OSM on the expression of membrane-bound TRAIL. **a** Decrease in the proportion of TME-infiltrating non-leukocyte cells after the addition of anti-CD3/CD28 Dynabeads and nivolumab. **b** OSM did promote an increase in CD45$^-$ TRAIL$^+$ cells isolated from the tumor tissue. The addition of nivolumab to OSM did not further increase the proportions of CD45$^-$ TRAIL$^+$ cells. **c** Nivolumab exhibited augmenting effects on the proportions of CD45$^-$ TRAIL$^+$ cells.

TIICs. However, in the tumor compartment, OSM promoted even more significant enrichment of cell culture with CD8$^+$ TIICs, suggesting promising efficacy of intratumoral administration of OSM.

Lastly, we examined whether OSM or nivolumab could affect the susceptibility of nonimmune cells (CD45$^-$ cells) to TRAIL-induced death. We observed that in the tumor compartment, OSM did not affect the proportion of CD45$^-$ cells but triggered an increase in CD45$^-$ TRAIL$^+$ cells.

Our data suggest that OSM may play a role in the treatment of leiomyosarcoma, myxofibrosarcoma, and liposarcoma. We demonstrated that the biological efficacy of OSM is reflected in the TME rather than in the peripheral blood of these patients, and nivolumab could potentiate its mechanism of action in selected cases. Nevertheless, more histotype-tailored studies are needed to fully understand the functions of OSM in STSs.

Our study has several limitations worth mentioning. The primary disadvantage of this study is the small number of the study participants. Although our results revealed uniform tendencies among STS patients, larger study cohorts need to be created to ensure the relevance of OSM agent in sarcoma treatment. Histology might be critical for choosing the appropriate therapy. For instance, five distinct subtypes of liposarcoma display major variations in the composition of the TME [29]. While

dedifferentiated liposarcoma became the predominant liposarcoma subtype evaluated in clinical trials with immune checkpoint inhibitors due to its relatively high infiltration with T cells, myxoid liposarcoma has become a prime target for CAR T-cell therapy [41]. Since a large portion of dedifferentiated liposarcomas derive from well-differentiated liposarcomas, we aimed to evaluate the immunotherapy responses in this particular liposarcoma subtype [41]. However, our limited cohort size hampered prediction of treatment response based on histological subtype of STS. Moreover, this study was initially designed to investigate the effects of OSM and nivolumab administration in vitro, and the observations were made in a single time point. Both the in vivo effects, as well as the long-term effects, should be taken in consideration when designing different studies evaluating the potential therapeutic role of OSM.


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## Statements of Ethics

The study was approved by the Ethics Committee of Motol University Hospital (EK-189/20) and carried out according to the Declaration of Helsinki of 1964 and its subsequent amendments. A written informed consent was obtained from all study participants.

## Conflict of Interest Statement

Jirina Bartunkova is a part-time employee and a minority shareholder of Sotio, a.s., a biotech company developing a cell-based immunotherapy. Giulia Toffanin, Andrej Ozaniak, Robin Bartolini, Michal Rataj, Rene Novysedlak, Jitka Smetanova, Martin Komarc, Antonio Rosato Robert Lischke, and Zuzana Strizova declare no conflict of interest.


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## Author Contribution

Conceptualization: G.T., A.O., and Z.S.; methodology: G.T. and Z.S.; formal analysis: Z.S., J.S., M.K., and M.R.; investigation: A.O. and Z.S.; resources: A.O. and Z.S.; software and statistics: M.K., Z.S., and J.S.; visualization: R.N., Z.S., and R.B.; writing – original draft: Z.S., R.B., A.O., and G.T.; supervision: Z.S., R.L., A.R., and J.B.; funding acquisition: J.B., R.L., Z.S., and A.R.; writing – review and editing: G.T., A.O., R.B., M.R., R.N., M.R., J.S., A.R., J.B., R.L., and Z.S.

## Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

## References


1 Ozaniak A, Vachtenheim J Jr, Lischke R, Bartunkova J, Strizova Z. Novel insights into the immunotherapy of soft tissue sarcomas: do we need a change of perspective? Biomedicines, 2021. 9(8):935.
2 Gamboa AC, Gronchi A, Cardona K. Soft-tissue sarcoma in adults: an update on the current state of -specific management in an era of personalized medicine. CA Cancer J Clin. 2020;70(3):200–29.
3 Gronchi A, Miah AB, Dei Tos AP, Abecassis N, Bajpai J, Bauer S, et al. Soft tissue and visceral sarcomas: ESMO-EURACAN-GENTURIS clinical practice guidelines for diagnosis, treatment and follow-up(☆). Ann Oncol. 2021;32(11):1348–65.
4 Ozaniak A, Smetanova J, Bartolini R, Rataj M, Capkova L, Hacek J, et al. A novel anti-CD47-targeted blockade promotes immune activation in human soft tissue sarcoma but does not potentiate anti-PD-1 blockade. J Cancer Res Clin Oncol, 2022.
5 Gronchi A, Ferrari S, Quagliuolo V, Broto JM, Pousa AL, Grignani G, et al. Histotype-tailored neoadjuvant chemotherapy versus standard chemotherapy in patients with high-risk soft-tissue sarcomas (ISG-STS 1001): an international, open-label, randomised, controlled, phase 3, multicentre trial. Lancet Oncol. 2017;18(6):812–22.
6 Stoletov K, Beatty PH, Lewis JD. Novel therapeutic targets for cancer metastasis. Expert Rev Anticancer Ther. 2020;20(2):97–109.
7 Edwards SC, Hoevenaar WHM, Coffelt SB. Emerging immunotherapies for metastasis. Br J Cancer. 2021;124(1):37–48.

8 Berraondo P, Sanmamed MF, Ochoa MC, Etxeberria I, Aznar MA, Perez-Gracia JL, et al. Cytokines in clinical cancer immunotherapy. Br J Cancer. 2019;120(1):6–15.
9 Naimi A, Mohammed RN, Raji A, Chupradit S, Yumashev AV, Suksatan W, et al. Tumor immunotherapies by immune checkpoint inhibitors (ICIs); the pros and cons. Cell Commun Signal. 2022;20(1):44.
10 Agostinetto E, Montemurro F, Puglisi F, Criscitiello C, Bianchini G, Del Mastro L, et al. Immunotherapy for HER2-positive breast cancer: clinical Evidence and future perspectives. Cancers. 2022;14(9):2136.
11 Houben E, Hellings N, Broux B. Oncostatin M, an underestimated player in the central nervous system. Front Immunol. 2019;10:1165.
12 Ma Y, Streiff RJ, Liu J, Spence MJ, Vestal RE. Cloning and characterization of human oncostatin M promoter. Nucleic Acids Res. 1999;27(23):4649–57.
13 Masjedi A, Hajizadeh F, Beigi Dargani F, Beyzai B, Aksoun M, Hojjat-Farsangi M, et al. Oncostatin M: a mysterious cytokine in cancers. Int Immunopharmacol. 2021;90:107158.
14 Juan TSC, Bolon B, Lindberg RA, Sun Y, Van G, Fletcher FA. Mice overexpressing murine oncostatin M (OSM) exhibit changes in hematopoietic and other organs that are distinct from those of mice overexpressing human OSM or bovine OSM. Vet Pathol. 2009;46(1):124–37.
15 Lilja A, Nordborg C, Brun A, Salford LG, Aman P. Expression of the IL-6 family cytokines in human brain tumors. Int J Oncol. 2001;19(3):495–9.
16 Koskela, K, Pelliniemi TT, Rajamaki A, Pulkki K, Remes K. Serum oncostatin M in multiple myeloma: impact on disease severity and prognosis, Eur J Haematol, 2000;65(1):p. 52–6,
17 Guo Q, Guan GF, Cao JY, Zou CY, Zhu C, Cheng W, et al. Overexpression of oncostatin M receptor regulates local immune response in glioblastoma. J Cel Physiol. 2019;234(9):15496–509.
18 Smith DA, Kiba A, Zong Y, Witte ON. Interleukin-6 and oncostatin-M synergize with the PI3K/AKT pathway to promote aggressive prostate malignancy in mouse and human tissues. Mol Cancer Res. 2013;11(10):1159–65.
19 Queen MM, Ryan RE, Holzer RG, Keller-Peck CR, Jorcyk CL. Breast cancer cells stimulate neutrophils to produce oncostatin M: potential implications for tumor progression. Cancer Res. 2005;65(19):8896–904.
20 Fossey SL, Bear MD, Kisseberth WC, Pennell M, London CA. Oncostatin M promotes STAT3 activation, VEGF production, and invasion in osteosarcoma cell lines. BMC Cancer. 2011;11:125.
21 David E, Guihard P, Brounais B, Riet A, Charrier C, Battaglia S, et al. Direct anti-cancer effect of oncostatin M on chondrosarcoma. Int J Cancer. 2011;128(8):1822–35.
22 Tanaka M, Miyahima A. Onconstatin M, a multifunctional cytokine. in: Reviews of Physiology, Biochemistry and Pharmacology. Berlin, Heidelberg: Springer Berlin Heidelberg; 2004. p. 39–52.
23 Li C, Ahlborn TE, Kraemer FB, Liu J. Oncostatin M-induced growth inhibition and morphological changes of MDA-MB231 breast cancer cells are abolished by blocking the MEK/ERK signaling pathway. Breast Cancer Res Treat. 2001;66(2):111–21.
24 Halfter H, Friedrich M, Resch A, Kullmann M, Stogbauer F, Ringelstein EB, et al. Oncostatin M induces growth arrest by inhibition of Skp2, Cks1, and cyclin A expression and induced p21 expression. Cancer Res. 2006;66(13):6530–9.
25 Jahani-Asl A, Yin H, Soleimani VD, Haque T, Luchman HA, Chang NC, et al. Control of glioblastoma tumorigenesis by feed-forward cytokine signaling. Nat Neurosci. 2016;19(6):798–806.
26 Yu Z, Li Z, Wang C, Pan T, Chang X, Wang X, et al. Oncostatin M receptor, positively regulated by SP1, promotes gastric cancer growth and metastasis upon treatment with Oncostatin M. Gastric Cancer. 2019;22(5):955–66.
27 Caffarel MM, Coleman N. Oncostatin M receptor is a novel therapeutic target in cervical squamous cell carcinoma. J Pathol. 2014;232(4):386–90.
28 Carbonnaux M, Brahmi M, Schiffler C, Meeus P, Sunyach MP, Bouhamama A, et al. Very long-term survivors among patients with metastatic soft tissue sarcoma. Cancer Med. 2019;8(4):1368–78.
29 Dancsok AR, Setsu N, Gao D, Blay JY, Thomas D, Maki RG, et al. Expression of lymphocyte immunoregulatory biomarkers in bone and soft-tissue sarcomas. Mod Pathol. 2019;32(12):1772–85.
30 Tawbi HA, Burgess M, Bolejack V, Van Tine BA, Schuetze SM, Hu J, et al. Pembrolizumab in advanced soft-tissue sarcoma and bone sarcoma (SARC028): a multicentre, two-cohort, single-arm, open-label, phase 2 trial. Lancet Oncol. 2017;18(11):1493–501.
31 D'Angelo SP, Mahoney MR, Van Tine BA, Atkins J, Milhem MM, Jahagirdar BN, et al. Nivolumab with or without ipilimumab treatment for metastatic sarcoma (Alliance A091401): two open-label, non-comparative, randomised, phase 2 trials. Lancet Oncol. 2018;19(3):416–26.
32 Fuss IJ, Kanof ME, Smith PD, Zola H. Isolation of whole mononuclear cells from peripheral blood and cord blood. Curr Protoc Immunol. 2009. Chapter 7:7.1.1-7.1.8.
33 Yasuda T, Koiwa M, Yonemura A, Akiyama T, Baba H, Ishimoto T. Protocol to establish cancer-associated fibroblasts from surgically resected tissues and generate senescent fibroblasts. STAR Protoc. 2021;2(2):100553.
34 Strizova Z, Taborska P, Stakheev D, Partlova S, Havlova K, Vesely S, et al. NK and T cells with a cytotoxic/migratory phenotype accumulate in peritumoral tissue of patients with clear cell renal carcinoma. Urol Oncol. 2019;37(7):503–9.
35 Valitutti S, Muller S, Salio M, Lanzavecchia A. Degradation of T cell receptor (TCR)-CD3-zeta complexes after antigenic stimulation. J Exp Med. 1997;185(10):1859–64.
36 Camacho M, Aguero A, Sumarroca A, Lopez L, Pavon MA, Aviles-Jurado FX, et al. Prognostic value of CD45 transcriptional expression in head and neck cancer. Eur Arch Otorhinolaryngol. 2018;275(1):225–32.
37 Ye N, Cai J, Dong Y, Chen H, Bo Z, Zhao X, et al. A multi-omic approach reveals utility of CD45 expression in prognosis and novel target discovery. Frontiers in Genetics. 2022;13. 928328.
38 Li L, Lu G, Liu Y, Gong L, Zheng X, Zheng H, et al. Low infiltration of CD8+ PD-l1+ T cells and M2 macrophages predicts improved clinical outcomes after immune checkpoint inhibitor therapy in non-small cell lung carcinoma. Front Oncol. 2021;11:658690.
39 Strizova Z, Snajdauf M, Stakheev D, Taborska P, Vachtenheim J Jr, Biskup J, et al. The paratumoral immune cell signature reveals the potential for the implementation of immunotherapy in esophageal carcinoma patients. J Cancer Res Clin Oncol. 2020;146(8):1979–92.
40 Snajdauf M, Havlova K, Vachtenheim J Jr, Ozaniak A, Lischke R, Bartunkova J, et al. The TRAIL in the treatment of human cancer: an update on clinical trials. Front Mol Biosci. 2021;8:628332.
41. Resag A, Toffanin G, Benešova I, Muller L, Potkrajcic V, Ozaniak A, et al. The immune contexture of liposarcoma and its clinical implications. Cancers. 2022;14(19):4578.