Spatial distribution of tumour immune infiltrate predicts outcomes of patients with high-risk soft tissue sarcomas after neoadjuvant chemotherapy

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Summary

Background **Anthracycline-based neoadjuvant chemotherapy (NAC) may modify tumour immune infiltrate. This study characterized immune infiltrate spatial distribution after NAC in primary high-risk soft tissue sarcomas (STS) and investigate association with prognosis.**

Methods **The ISG-STS 1001 trial randomized STS patients to anthracycline plus ifosfamide (AI) or a histology-tailored (HT) NAC. Four areas of tumour specimens were sampled: the area showing the highest lymphocyte infiltrate (HI) at H&E; the area with lack of post-treatment changes (highest grade, HG); the area with post-treatment changes (lowest grade, LG); and the tumour edge (TE). CD3, CD8, PD-1, CD20, FOXP3, and CD163 were analyzed at immunohistochemistry and digital pathology. A machine learning method was used to generate sarcoma immune index scores (SIS) that predict patient disease-free and overall survival (DFS and OS).**

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Findings Tumour infiltrating lymphocytes and PD-1+ cells together with CD163+ cells were more represented in STS histologies with complex compared to simple karyotype, while CD20+ B-cells were detected in both these histology groups. PD-1+ cells exerted a negative prognostic value irrespectively of their spatial distribution. Enrichment in CD20+ B-cells at HI and TE areas was associated with better patient outcomes. We generated a prognostic SIS for each tumour area, having the HI-SIS the best performance. Such prognostic value was driven by treatment with AI.

Interpretation The different spatial distribution of immune populations and their different association with prognosis support NAC as a modifier of tumour immune infiltrate in STS.

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Keywords: Soft tissue sarcomas; Tumour immune microenvironment; Neoadjuvant chemotherapy; Anthracycline

Research in context

Evidence before this study

In the context of soft tissue sarcomas (STS), the ISG-1001 randomized controlled trial (RCT) conducted a comparison between anthracycline plus ifosfamide (AI) and histology-tailored (HT) neoadjuvant chemotherapy (NAC). The results of this study seem to support the AI for patients deemed at high risk of mortality.

STS are often characterized by a limited immune infiltrate. In diverse cancer types, NAC is recognized for its capacity to influence the tumour immune microenvironment (TIME), thereby augmenting the efficacy of immune checkpoint inhibitors (ICI). Although there is existing evidence in the realm of STS that underscores the influence of radiotherapy on the TIME, a comprehensive understanding of the implications of NAC remains to be fully elucidated.

Added value of this study

This study was carried out as part of the aforementioned trial and focused on examining the spatial distribution of tumour immune infiltrate in four specific regions of primary high-risk STS following NAC.

Implications of all the available evidence

The composition of the immune microenvironment following NAC plays a crucial role in stratifying patient risk. Patients with tumours characterized by high PD-1 expression across the entire tumour or low CD20 expression at the tumour's periphery, especially in areas with the richest immune infiltrate, are at a high risk.

Notably, NAC with AI appears to influence these prognostic associations, suggesting a potential impact of AI on the immune microenvironment of STS. These findings provide a strong rationale for the development of a clinical trial that combines immune ICIs and AI for neoadjuvant treatment in patients with high-risk primary STS located in the extremities or trunk wall.

Introduction

Anthracyclines are used for their cytotoxic anti-cancer activity and act as a modifier of tumour immune microenvironment (TIME) in several solid tumours.[1–6] These agents exert their immune-modulation effects through several mechanisms, which include depletion of myeloid-derived suppressor cells,[7] induction of tertiary lymphoid structures (TLS),[8–10] increase of type I interferon level,[11] activation of the complement[12] and immunogenic cell death.[13–15]

In soft tissue sarcomas (STS), a randomized controlled trial (RCT, ISG-1001[16,17]) compared anthracycline plus ifosfamide (AI) and histology-tailored (HT) neoadjuvant chemotherapy (NAC) in the commonest STS types and suggested that AI should remain the regimen to choose when NAC is considered in patients with high-risk STS according to the prognostic tool Sarculator.[16,18–20] STS have been considered tumours characterized by a relatively low immune infiltrate reflecting their low tumour mutation burden (TMB)[21,22] and composition of TIME shows variations across sarcoma histologies.[23–32] Consequently, immune checkpoint inhibitors showed some efficacy in metastatic patients with selected sarcoma histologies[25,33–41] or specific TIME features, such as the presence of TLS.[42] We hypothesized that anthracycline-based NAC may act as modifier of TIME in high-risk STS and conducted a planned translational study of the above mentioned ISG-1001 RCT[16,17] to characterize the spatial distribution of tumour immune infiltrate after NAC and investigate association with patient risk.

Methods

Patients

This was a pre-planned translational study of the ISG-STS 1001 clinical trial (ClinicalTrials.gov ID: NCT01710176; European Union Drug Regulating Authorities Clinical Trials database ID: 2010-023484-17).[16,17] The study protocol and all amendments were approved by the appropriate independent ethics committee at each trial centre. The ISG-1001 was conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent before enrolment. The study design was reported elsewhere[16,17] and is summarized in online available Supplementary material. Reporting of this manuscript met the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement.

Spatial detection and quantification of immune cells

Tissue MicroArray (TMA) were generated sampling both pre-treatment biopsies and post-operative specimens. Pre-treatment biopsy where selected when formalin-fixed paraffin-embedded (FFPE) were left after diagnosis of other translational studies planned in the ISG-1001. Surgical specimens were selected when residual stainable tumour was present after surgery. In pre-treatment biopsy, two 1 mm cores were punched from FFPE. In surgical specimen, the area for TMA sampling was selected after revision of the original slides by a soft-tissue pathologist and haematoxylin and eosin (H&E) stained sections were prepared. Four areas of the surgical specimens were sampled (Fig. 1A) according to the study protocol whenever possible as follow:

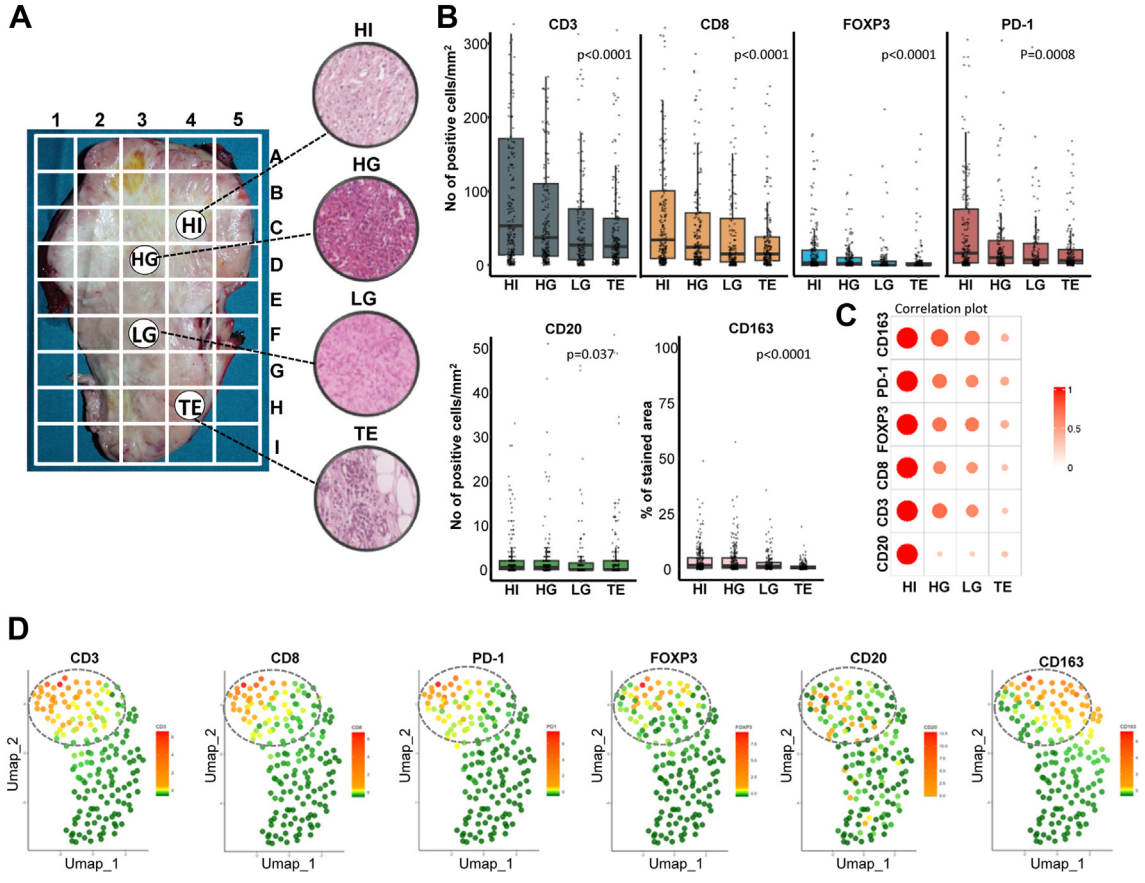

Fig. 1: **Tumour immune microenvironment (TIME) of patients with high-risk soft tissue sarcomas after neoadjuvant chemotherapy (N = 246).** After neoadjuvant chemotherapy and surgery, the following four tumour areas were sampled at histopathological examination: 1) the area showing morphologically highest lymphocyte infiltrate (HI) at H&E; 2) the highest grade (HG) stainable tumour representing the area with lack of post-treatment changes; 3) the lowest grade (LG) stainable tumour representing the area with post-treatment changes; 4) the tumour edge (TE), defined as the area within 1 mm from tumour border (**A**). There was a progressive and statistically significant decrease of the T cell subsets from HI to TE, while no major difference was observed in the distribution of CD20+ B cells and CD163+ macrophages (**B**). The presence of immune infiltrate was consistent within the different cores in each tumour, as indicated by the statistically significant correlation observed for all immune cell subsets (**C**). In tumours characterized by a high number of immune cells in their microenvironment, T cell subsets appeared to be intimately associated with macrophages and B cells, co-localizing in a selected patient cluster according to UMAP analysis (**D**).

1. One 1 mm core from the area showing morphologically highest lymphocyte infiltrate (HI) at H&E;
2. Three 1 mm cores from the highest grade (HG) stainable tumour representing the area with lack of post-treatment changes (i.e., high number of residual stainable tumour cells[43]);
3. One 1 mm core from the lowest grade (LG) stainable tumour representing the area with post-treatment changes (i.e., low number of residual stainable tumour cells[43]);
4. Two 1 mm cores from the tumour edge (TE), defined as the area within 1 mm from the tumour border.

Detailed information on TMA, immunohistochemistry (IHC) and digital pathology[44] are reported in online available Supplementary material.

Statistical analysis

Firstly, statistical analysis was performed to test the association between investigated immune cell populations with the following tumour and treatment features: i) STS histology, comparing STS characterized by histologies with a complex karyotype [ck-STS, including undifferentiated pleomorphic sarcoma (UPS), malignant peripheral nerve sheath tumour (MPNST) and leiomyosarcoma (LMS)] to those with a simple karyotype [sk-STS, such as synovial sarcoma (SS) and high-grade myxoid liposarcoma (HG-MLPS)]; ii) NAC, comparing AI and HT schedules; and iii) neoadjuvant radiotherapy (RT), comparing STS treated or not with preoperative RT.

Secondly, an explorative analysis was conducted in patients who had paired pre-treatment tumour biopsy and post-operative surgical specimen to assess the modulation of infiltrating immune cells induced by NAC.

Thirdly, multivariable models were built exploiting AIM, which is a machine learning method incorporating variable selection and dichotomization,[45] to generate sarcoma immune index scores (SIS) that predict patient disease-free and overall survival (DFS and OS).[46] DFS was defined as the time from randomization to either local recurrence or distant metastasis, while OS was defined as the time from randomization to patient death for any cause. Description of statistical analysis[44,47] is included in online available Supplementary material.

Results

Overall, 246 over 435 study patients were included in this analysis (CONSORT diagram, clinic-pathological features and follow-up data are reported in Supplementary Figure S1 and Table 1, respectively).

Distribution of immune cell infiltrate in STS after NAC

The four tumour areas that were sampled for each study patient showed a progressive and statistically significant decrease of all investigated cell subsets from HI to TE (Fig. 1A and B). CD3+ and CD8+ T-cells were the most represented populations in all four areas; PD-1+ cells were also abundant, suggesting the presence of exhausted effectors. Similarly, the distribution of CD20+ B-cells, which differed although to a lesser extent, were scantly, albeit detectable (≤1 cell), in a relevant

Clinico-pathological characteristics	Neoadjuvant AI		Neoadjuvant HT	
	No.	%	No.	%
Age (years)	Median 53 (IQR, 42.5–60.5)		Median 51 (IQR, 41.5–58.5)	
Sex				
Female	63	0.38	28	0.35
Male	104	0.62	51	0.65
Size (mm)	Median 110 (IQR, 80–149.5)		Median 97 (IQR, 75–137.5)	
Sarcoma histology				
HG-MLPS	36	0.22	18	0.23
UPS	40	0.24	28	0.35
SS	19	0.11	18	0.23
LMS	14	0.08	9	0.11
MPNST	9	0.05	6	0.08
Others	49	0.29	0	0
Neoadjuvant RT				
Not performed	109	0.65	69	0.87
Performed	58	0.35	10	0.13

AI: anthracycline + ifosfamide; HT, histology-tailored chemotherapy; No., number; IQR, interquartile range; HG-MLPS, high grade myxoid liposarcoma; UPS, undifferentiated pleomorphic liposarcoma; SS, synovial sarcoma; LMS, leiomyosarcoma; MPNST, malignant peripheral nerve sheath tumor; RT, radiotherapy.

Table 1: **Clinical and pathological features of included patients.**

proportion of patients. The presence of immune infiltrate was consistent within the different cores in each tumour, as indicated by the significant correlation between immune cell subsets (Fig. 1C and Supplementary Figure S2). T-cell subsets appeared to be intimately associated with B-cells and CD163+ tumour associated macrophages (TAM), co-localizing in a selected patient cluster (Fig. 1D).

In all the four tumour cores, expression of T-cell subsets, including PD-1+ cells, and CD163+ TAM was higher in ck-STS compared to sk-STS (Fig. 2A). Conversely, the number of CD20+ B-cells appeared independent of tumour karyotype.

With the exception of CD163+ TAM, no quantitative differences in the level of infiltrating T- and B-cells in patients who received neoadjuvant AI compared to patients treated with neoadjuvant HT were observed (Fig. 2B). Neoadjuvant RT lowered CD3+, CD8+ and PD-1+ cells compared to patients who had NAC alone (Fig. 2C).

We performed an explorative analysis in a limited number of patients who had matched pre-treatment tumour biopsy and post-operative surgical specimen available (N = 13), showing association of AI with an increase of CD3+, CD8+, PD-1+, and CD163+ cells (Supplementary Figure S3).

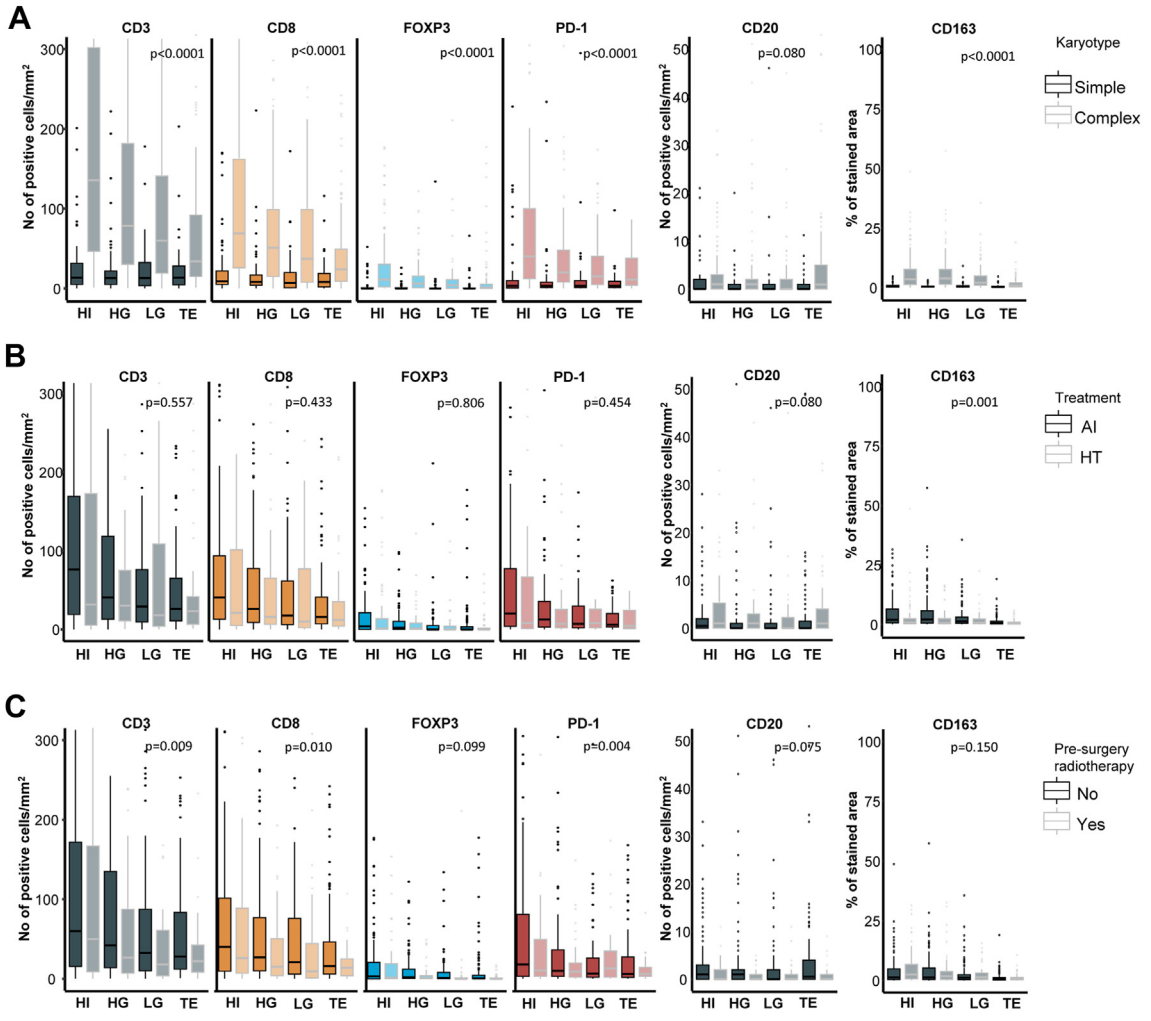

Fig. 2: **Quantitative evaluation of tumour immune microenvironment (TIME) of patients with high-risk soft tissue sarcomas (STS).** In the four tumour cores, T cell subsets, including PD1+ cells, and CD163+ cells were expressed at a statistically significant higher number in complex karyotype (ck)-STS (N = 155) compared to simple karyotype (sk)-STS (N = 91), while number of CD20+ B cells appears to be independent of tumour karyotype (**A**). The following STS were labelled as sk-STS: HG-MLPS and SS. The following STS were labelled as ck-STS: UPS, LMS, MPNST, myxofibrosarcoma, pleomorphic liposarcoma, pleomorphic rhabdomyosarcoma and unclassified spindle cell sarcoma. With the exception of CD163+ macrophages, level of infiltrating T cells and B cells in patients who received neoadjuvant AI (N = 167) or HT (N = 79) did not differ statistically (**B**). Number of infiltrating CD3+, CD8+ and PD-1+ cells is higher in the TIME of patients with high-risk STS treated with neoadjuvant radiation (N = 68) compared to TIME of patients with STS that did not receive neoadjuvant radiation (N = 176) (**C**).

Identification of prognostic sarcoma immune index scores (SIS) in spatially distinct TMA cores

Key information on the SIS built on the level of immune markers in each tumour area (i.e., HI, HG, LG, and TE) is provided in Supplementary Table S1. The stability of each SIS is determined by multiple results obtained through iterative analysis (Supplementary Table S2) which resulted in different weights for each immune cell population (Supplementary Figure S4).

Overall, PD-1+ (above cut-off) and CD8+ and CD3+ T-cells (below cut-off) were consistently selected in all the scores, while the CD20+ B-cells (below cut-off) was highly represented in HI and TE areas (Supplementary Table S1). CD163+ TAM (above cut-off) was selected only at TE. Of note, the cut-off values of CD20+, FOXP3+ (Treg) and CD163+ cells were generally low in all tumour areas, suggesting that a quantitatively marginal presence of B-cells, T-reg and TAM may influence disease course and patient outcomes. A comprehensive description of the immune cell populations included in each SIS together with their cut-off values at the single patient level is reported in Supplementary Figure S5.

In the HI area, the HI-SIS included CD8+, CD20+ and PD-1+ cells, with a prevalent role of low CD8+ cells (below cut-off) and a comparable contribution of CD20+ (below cut-off) and PD-1+ (above cut-off; Supplementary Figure S5). This score demonstrated the best stability among the analyzed scores.

In the HG zone, the HG-SIS included CD3+ and PD-1+ cells, which were consistently selected, with PD-1 being chosen in every iteration and CD3 in 86% of the cases. However, the CD3 cut-off value exhibited high variance and resulted in low precision of its prognostic value.

In the LG area, potentially representing the residual stainable tumour characterized by post-treatment changes, SIS encompassed a more complex immune marker panel, including CD8+, PD-1+, FOXP3+ and CD3+ cells. This evidence indicates that tumour control might require activated non-exhausted T-cells and a limited impact of Treg. These selected markers demonstrated a high number of occurrences across all iterations and the precision of their cut-off values was high, indicating their significance in the prognostic model. However, this score was calculated on a relatively low number of patients (N = 153) due to technical issues with generating TMA from tumours with meaningful post-treatment changes.

Finally, the TE-SIS included the broadest array of immune cell subsets, including T-, B-cells and, for the first time, CD163+ TAM, with a comparable contribution of all markers except CD8+ T-cells, which was apparently less relevant at this site. However, the inclusion of a higher number of markers makes the score more complex. Notably, the CD3 marker shows a considerable variance in its cut-off values, making it unreliable for prognostic assessment.

Association of the prognostic sarcoma immune index scores (SIS) with patient DFS and OS

The SIS for the different tumour areas displayed a remarkable association with patient DFS (Fig. 3). Indeed, when grouped in two or three clusters based on the score complexity, patients with low SIS values showed overall a better DFS with respect to patients with high scores (Fig. 3). HI-SIS, which was built on the statistically most stable CD8+, PD-1+ and CD20+ cells, clustered patients in two balanced groups (N = 93 for score $\leq$1 vs N = 132 for score >1) with DFS curves diverging at 12 months and reaching a median DFS of 82 and 45 months, respectively (log-rank test, $P < 0.001$). DFS maintained statistically significant differences when adjusted for patient risk according to Sarculator nomogram score (Supplementary Table S3). Patients clustering according to three remaining SIS groups also showed convincing association with DFS, particularly for LG-SIS (log-rank test, $P < 0.001$) and TE-SIS (log-rank test, $P < 0.001$). The SIS-HG score, possibly reflecting a poorly effective immune infiltrate in the 'non-responding' tumour area, showed instead a weaker, although statistically significant, correlation (log-rank test, $P = 0.033$) compared to the other SIS scores.

Analysis of OS showed that the HI-SIS score confirmed a statistical significance association with OS, while the remaining three scores did not show convincing associations with OS (Supplementary Figure S6; Supplementary Table S4).

Finally, differences between NAC schedules were investigated to evaluate whether anthracycline-based NAC may drive the prognostic value of the SIS scores. The HI-SIS and TE-SIS were significantly associated to DFS after AI (log-rank test, $P < 0.001$ and $P = 0.001$, respectively) but not after HT chemotherapy (log-rank test, $P = 0.65$ and $P = 0.086$, respectively; Fig. 4). AI resulted in significant prognostic value of SIS also in the area with most pronounced post treatment changes (LG, log-rank test, $P < 0.001$) compared to HT chemotherapy (log-rank test, $P = 0.062$). As expected for the HG area, the association of HG-SIS score with DFS for either AI or HT chemotherapy did not reach statistical significance (log-rank test, AI: $P = 0.18$, HT: AI: $P = 0.18$; Supplementary Figure S7).

Discussion

This study investigated the spatial distribution of tumour immune infiltrate in high-risk STS treated with AI or an HT NAC followed by surgery with or without RT in a RCT. We analyzed four different tumour areas after NAC, characterized by the richest tumour infiltrate at H&E (HI), high and low grade (HG and LG) according to histopathological response to NAC,[43,48] and the tumour edge (TE). Quantitative differences in distribution of immune populations in the different STS areas

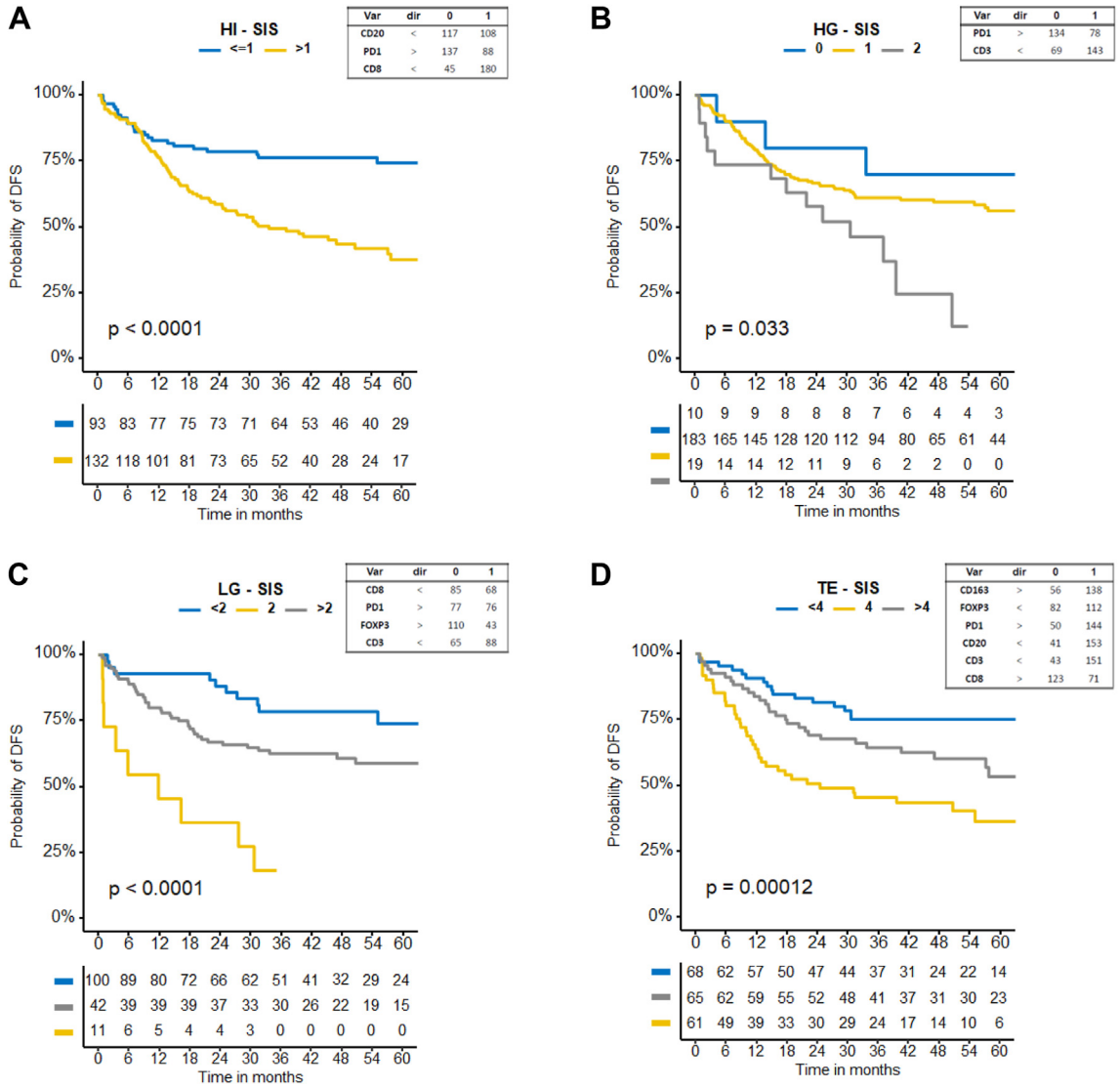

***Fig. 3*: The Sarcoma index score (SIS) at each of the four sampled tumour areas: composition of the score and association with patient disease-free survival (DFS).** Composition of the SIS in each tumour area and direction of the association with patient DFS. Each marker scores a point (+1) when detected above/below the cut-off, according to the direction of the association. Kaplan-Meier survival curves for DFS of the SIS at each tumour area. *P*-values of log-rank tests are reported in each figure. Highest lymphocyte infiltrate (HI; **A**); highest grade (HG; **B**); lowest grade (LG; **C**); tumour edge (TE; **D**).

and, most importantly, their different association with patient prognosis support the hypothesis that NAC may act as a modifier of TIME in STS. In addition, such hypothesis is reinforced by the observation that this prognostic association holds in patients treated with AI but not with HT NAC, a group of chemotherapy schedules that did not include an anthracycline. Overall, these findings provide the background to design a clinical trial to investigate the addition of immune checkpoint inhibitors to NAC with anthracyclines in patients with high-risk STS.

The evidence generated by this study is limited by the small number of patients with available both pre-treatment biopsy and post-surgical specimen as these biopsies were primarily utilized for diagnostic purposes and in other translational studies associated with this trial.[49,50] However, a higher tumour immune infiltrate was detected after AI when both pre-treatment biopsy and tumour specimen were available (N = 13). Furthermore, this study examined the correlation between the composition of TIME following NAC and patient outcomes. It is important to acknowledge that

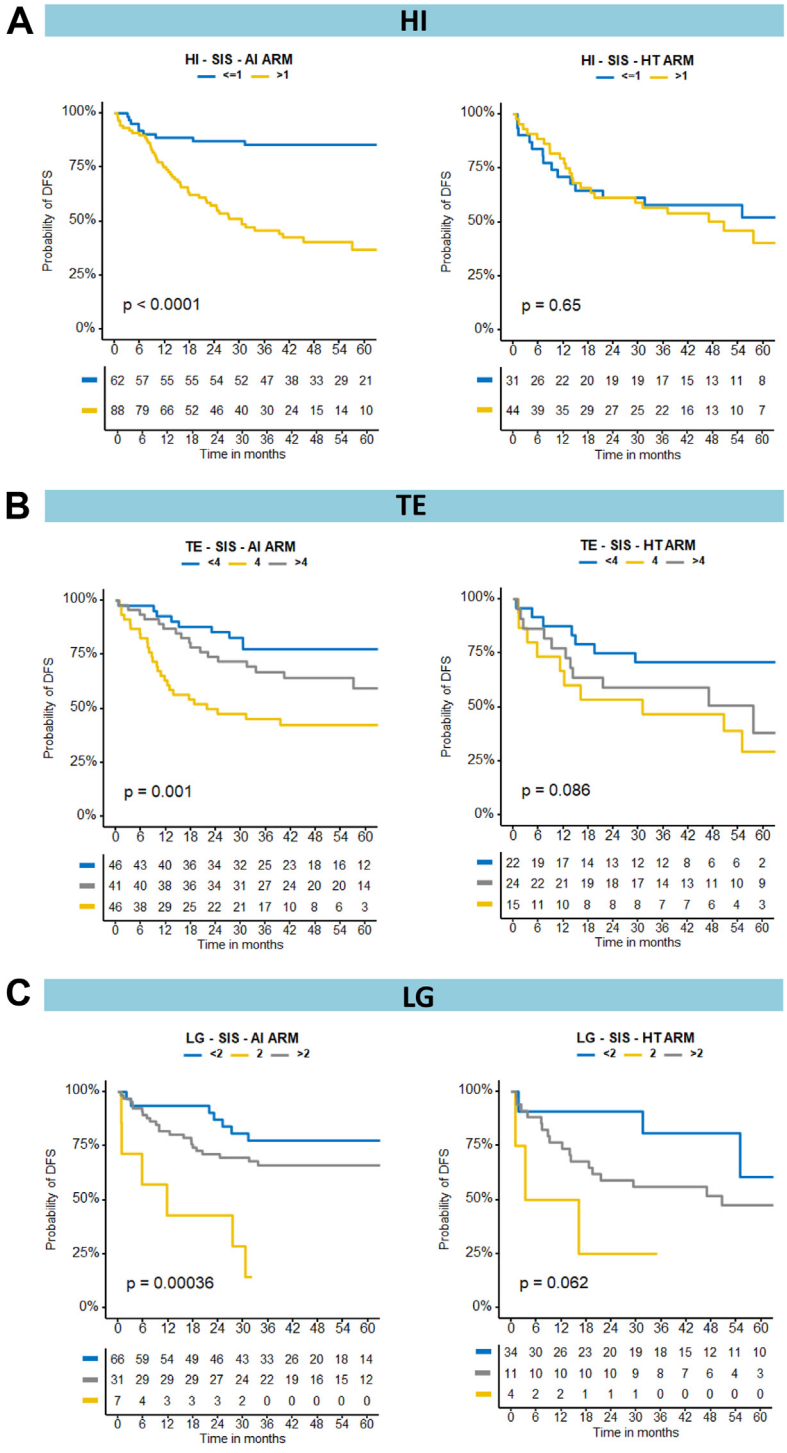

Fig. 4: **The association of the sarcoma index score (SIS) with prognosis is driven by treatment with neoadjuvant anthracycline plus ifosfamide (AI).** The Kaplan-Meier survival curves visualize the association of the HI-SIS (**A**), the TE-SIS (**B**), and the LG-SIS (**C**) according to whether patients were treated with anthracycline plus ifosfamide (AI, left column) or histology-tailored (HT) chemotherapy (HT, right column). *P*-values of log-rank tests are reported in each figure.

functional validation regarding the specific effects of anthracyclines on the TIME of STS was not included in these investigations. Additionally, findings of this study may be limited by issues with multiple comparisons that carry a risk of inflating false positive results. Although this study included multiple STS histologies ($N = 8$) and did not focus on a specific STS, these are the most frequently detected sarcomas in the extremities and superficial trunk wall and are commonly grouped together in clinical trials investigating perioperative therapies for primary localized high-risk patients.[16] Whilst this study investigated a relatively low number of immunological markers, it was designed to interrogate the TIME of STS patients treated with NAC to capture the key infiltrating cells that may influence patient outcomes and possibly add new prognostic information. We showed that the concerted triggering of both adaptive and innate immunity, possibly driven by B-cells,[51] is apparently required to mediate clinically effective tumour control. This concept confirms and extends the prognostic role of TLS in STS,[9,27,42] albeit the use of TMA did not formally allowed TLS quantification in our case sets.[52] Interestingly, T-cells and TAM were more represented in ck-STS compared to sk-STS, which is consistent with the neoantigen nature of immunological determinants recognized by T-cells.[53] Conversely, CD20+ B-cells appeared independent of STS karyotype group,[27,54] likely thanks to their ability of detecting self-proteins[51] and possibly promoting antitumour immunity even in cancers with reduced neoantigen repertoire.

Following treatment with NAC, we detected the concerted presence of effector (i.e., CD8+ T-cells and CD20+ B-cells) as well as exhausted and regulatory (i.e., PD-1+ cells, T-reg, and TAM) cells, supporting the induction of a full-fledged antitumour immune response, which is generally associated to the engagement of homeostatic mechanisms aimed at restraining damage in normal tissue. However, this effect appears to play a detrimental role on immune-mediated disease control as showed by the association of PD-1+ cells, Treg cells and TAM infiltrate with shorter DFS. Indeed, it could be hypothesized that strategies aimed at antagonizing regulatory immune pathways, such as PD-1 blockade, could contribute to prolong the benefit of NAC-induced immune responses in STS patients. This evidence support a clinical trial to test the implementation of immune checkpoint inhibitors together with NAC for patients with high-risk STS.

Spatial immunohistochemical data were gathered together in a prognostic sarcoma immune index score (SIS) for each tumour area, having the HI-SIS score the best performance. In the HI-SIS, patients with low expression of CD8+ T-cells and B-cells were at higher risk of developing disease recurrence after NAC plus surgery, a condition that was worsened by PD-1+ cells which suggested the presence of exhausted T-cells. The prognostic value of the HI-SIS was detected only after NAC with AI, supporting a functional modulation of TIME in high-risk STS after anthracycline-based NAC. In addition, in areas of tumours with lack of histopathologic response to NAC, here identified as the HG area, the negative prognostic effect of PD-1+ cells was coupled with that of CD3+ T-cells, suggesting that additional immune populations, such as CD4+ T-cells could influence risk of disease progression. The characterization of cell populations at the TE showed that presence of non-exhausted T-cells and lack of effect from Treg cells may result in a favourable prognostic scenario for patients treated with NAC and surgery. Conversely, TAM detected at the TE did associate with a higher risk, which is in keeping with previous reports on the detrimental effect of these cells in sarcomas[55] and at the invasive front in multiple solid cancers.[56–58] Our study confirmed the prognostic relevance of CD20 B cells detected at the TE.[9,27,42] Indeed, Petiprez et al. evaluated TLS at the tumour edge and demonstrated the association of mature TLS with longer patients survival and chances to develop a tumour response after treatment with an immune checkpoint inhibitor.[27]

This study has implications for future clinical research in STS. Firstly, it shows that anthracycline may modulate immune infiltrate in these tumours, a condition that has been investigated before in other solid tumours, such as breast cancer.[1,7,11,13] Secondly, our study identified PD-1 as a negative prognostic factor and a possible therapeutic target in primary high-risk STS treated with neoadjuvant AI. Consistently, patients who benefitted from pembrolizumab in the SARC0028 trial[33] were mostly affected by ck-STS (i.e., UPS or DDLPS) and had higher activated T-cells characterized by PD-1 expression, TAM characterized by PD-L1 expression, densities of effector memory cytotoxic T-cells and regulatory T-cells compared with non-responders.[59] In addition, this finding is in keeping with a retrospective study on primary STS after neoadjuvant therapy, where PD-1+ cells were associated with shorter survival.[60] An independent validation of the prognostic value of PD-1+ cells in patients with high-risk STS treated with NAC is needed.

Although we generated prognostic scores at each tumour area, the SIS score created in the HI area seems the best candidate for an independent validation. Remarkably, the HI area could be accurately identified by pathologists compared to HG and LG areas, the latter being also burdened by technical issue with TMA generation for the presence of post-chemotherapy changes. Computational analysis suggested that both the selected markers and their cut-offs for the HI-SIS were more reproducible compared to the scores achieved at the remaining three areas. In addition, fewer markers ($N = 3$) were selected by the HI score compared to the TE score ($N = 6$), easing the possible application of the HI score.

Finally, this study may have relevance also for the identification of patients who are more likely to have a

benefit after neoadjuvant AI. Indeed, the HI-SIS seems specifically related to treatment with AI. The relevance of this findings is related to the conflicting results of studies investigating the association between histopathological response and patient outcomes.[43,48,61]

In conclusion, this study shows the prognostic relevance of PD-1+ cells when analyzed in different areas of selected high-risk STS of extremities or trunk wall treated with NAC. CD20+ B cells correlate with better survival after chemotherapy at HI and TE areas. Prognostic risk stratification of immune infiltrate was observed when patients were treated with anthracyclines suggesting the possible role of this class of agents in modulating TIME. Although validation of these findings through functional experiments and analyses of independent cohort are needed to confirm these findings, the analyses here conducted support the design of a clinical trial that combine an immune checkpoint inhibitor and AI for the treatment of patients with high-risk STS of extremities or trunk wall.

Contributors

Study conception and design: Pasquali, Vallacchi, Collini, Gronchi.

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Data sharing statement

Deidentified participant data included in this manuscript will be made available to others upon request. A signed data access agreement will be required.

Declaration of interests

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2024.105220.

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