B cells are associated with survival and immunotherapy response in sarcoma

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First paragraph (185 words)

Soft-tissue sarcomas (STS) represent a heterogeneous group of cancer, with over 50 histologic subtypes[1,2]. They show variable clinical behaviours and response to therapy, including immune checkpoint blockade[3,4]. To explain this clinical variability, we studied gene expression profiles in 608 tumours across STS subtypes, establishing an immune-based classification based on the composition of the tumour microenvironment and identified five distinct phenotypes: immune-low (A, B), immune-high (D, E), and highly vascularised (C) groups. In situ analysis of an independent validation cohort showed that class E was also characterized by the presence of tertiary lymphoid structures (TLS) containing T cells, follicular dendritic cells and particularly rich in B cells. B cells are the strongest prognostic factor even in the context of high or low $CD8^+$ and cytotoxic contents. The immune-high E group demonstrated improved survival and a high response rate to PD-1 blockade by pembrolizumab in a phase 2 clinical trial. Altogether, this work provides novel evidence regarding the presence of immune subtypes in STS patients, and unravels the potential of B cell rich TLS to guide clinical decision-making and treatment, which could have broader applications beyond STS.

Main text (2210 words)

Soft-tissue sarcomas (STS) comprise many histologic subtypes with distinct clinical and biological behaviours. Genetically "simple" STS are characterized by translocations resulting in fusion-proteins and few if any other genomic lesions, while "complex" STS have an unbalanced karyotype and multiple genomic aberrations[1]. STS are considered "non-immunogenic" with low mutational burden[2]. Among complex tumours, undifferentiated pleomorphic sarcoma (UPS), dedifferentiated liposarcoma (DDLPS), and to a lesser extent leiomyosarcoma (LMS), can exhibit durable responses to immune checkpoint blockade, whereas simple tumours do not respond to PD-1 monotherapy or combination anti-PD1/anti-CTLA4[3,4]. Few reports investigating the composition of the tumour microenvironment (TME) composition in different STS histologies have been published[5–7], but a recent study from the Cancer Genome Atlas (TCGA) consortium suggested an association with prognosis[8].

Here, we developed a novel classification of STS, based on the composition of the tumour microenvironment in large cohorts of STS, using the MCP-counter method[9]. We found that the B lineage signature, a hallmark of an immune-high class we called E, correlated with an improved survival of STS patients, in tumours with both high or low $CD8^+$ T cells infiltration. In an independent cohort, we validated by immunohistochemistry the high density of B cells and presence of tertiary lymphoid structures (TLS) in class E. Finally, we showed that class E exhibited the highest response rate to PD-1 blockade therapy and improved progression-free survival in a multi-centre phase 2 clinical trial of pembrolizumab in STS (SARC028)[4,10].

RESULTS

Immune classification of STS: Histology-independent microenvironment composition and functional orientation

The TME compositions from 4 independent discovery primary STS datasets (TCGA SARC, GSE21050, GSE21122 and GSE30929, Extended Data Table 1) with publicly available gene expression profiles were analysed with MCP-counter, a gene-expression-based TME deconvolution tool[9]. An immune-based classification of STS was developed from this analysis (Methods, Extended Data Fig. 1) and tumours were assigned to one of five Sarcoma Immune Classes (SICs), labelled A, B, C, D and E, with highly distinct profiles (Fig. 1). We compared the SIC distribution across histologic subtypes and found that most LMS tumours were classified to SICs A and B (Fig. 1a). DDLPS accounted for half of SIC C tumours. Tumours classified as SICs D and E were more evenly distributed across histologic subtypes. Application of the predictor of the immune classes (Methods) to other STS histologies from French Sarcoma Group (FSG) cohort (Extended Data Table 1) revealed that all SICs could be identified in each histology (Extended Data Fig. 2a).

The TME composition differs significantly between SICs (Fig. 1b). Three SICs showed homogeneous profiles. SIC A, “immune desert”, was characterised by the lowest expression of gene signatures related to immune cells, as well as low vasculature. SIC C, “vascularised”, was dominated by a high expression of endothelial cell-related genes. SIC E, “immune and TLS high”, was characterized by the highest expression of genes specific to immune populations such as T cells, $CD8^+$ T cells, NK cells, cytotoxic lymphocytes. Strikingly, a key determinant of SIC E was the high expression of the B lineage signature (p=1.8e-29). SICs B and D were characterized by heterogeneous but generally “Immune low” and “Immune high” profiles, respectively.

Expression of genes associated with T cell/myeloid cell chemotaxis, T cell activation and survival, expression of major histocompatibility complex (MHC) class I, and regulatory gene signatures were high in SICs D and E, intermediate in SICs B and C, and very low in SIC A (Fig. 1c). Expression of the lymphoid structures-associated B cell chemo-attractant chemokine

CXCL13 was remarkably high in E tumours, moderate in D tumours, generally low in B and C tumours, and negligible in A tumours.

Immune checkpoint-related genes expression (Fig. 1d) followed that of immune infiltrates, with high expression of genes encoding PD-1, PD-L2, CTLA-4 and TIM3 (*PDCD1*, *PDCD1LG2*, *CTL4* and *HAVCR2*, respectively) in SIC E followed by SIC D tumours, and low to very low expression in SIC C, B and A tumours. *CD274* (PD-L1) was heterogeneously expressed across SICs while *LAG3* was only expressed at high levels in SIC E tumours with low expression in all other classes. The above findings were consistent across the four discovery cohorts (Extended Data Fig. 3).

SICs are associated with survival of STS patients

After verification that the two cohorts with available survival data (TCGA SARC, n=213; GSE21050, n=283) exhibited similar survival patterns (data not shown), these were pooled to study the clinical outcome of the five SICs (Fig. 2a). SIC A patients exhibited the shortest overall survival (OS) compared to D or E patients (p=0.048 and p=0.025, respectively). Similarly, among the other STS histologies from FSG cohort, SIC A patients had a shorter OS compared to SIC E patients (Extended Data Fig. 2b). In a multivariate model with classical prognostic factors (Fig. 2b), SICs were found to be significantly associated with prognosis, independent of other clinical parameters (as compared to SIC A, p=0.011 and p=0.029, for SIC D and SIC E, respectively). Tumours were separated between high and low expression of $CD8^+$ T cells, cytotoxic lymphocytes and B lineage signatures based on the observation of the MCP-counter scores distribution (Extended Data Fig. 4). Detailed analysis of the impact of these immune cell population signatures revealed that whereas neither $CD8^+$ T cells (Fig. 2c, p=0.277) nor cytotoxic lymphocytes (Extended Data Fig. 5a, p=0.0513) significantly correlated with survival, the B lineage signature was significantly associated with improved overall survival (Fig. 2d, p=4.25e-04). When analysed in the context of high or low infiltration by $CD8^+$

T cells (Fig. 2e), cytotoxic lymphocytes or expression of *PDCD1* (PD-1), *CD274* (PD-L1) or *FOXP3* (Extended Data Fig. 5b-e), the B lineage signature was the dominant parameter for improved survival, regardless of other immune factors expression. Additionally, SIC E tumours demonstrate high expression of both *IGJ* and *TNFRSF17* (encoding BCMA) (data not shown), indicating that plasma cells[11] may contribute to improve prognosis.

Mutational landscape of SICs in TCGA SARC

Overall Tumour Mutational Burden (TMB) was low across the studied cohorts (median: 32 non-synonymous mutations [NSM]) and appeared to be similar across all SICs (Extended Data Fig. 6a). However, a few highly mutated tumours (each with over 250 NSM) were found in the D and E groups. Qualitative mutational analysis revealed several commonly mutated genes across the cohort, including *TP53* (35.2%), *ATRX* (16.0%), *TTN* (9.9%), *RB1* (8.9%), *MUC16* (8.0%), *PCLO* (6.1%), *DNAH5*, *MUC17* and *USH2A* (5.2% each) (Extended Data Fig. 6b). *TP53* was more frequently mutated among SICs D and E tumours (Extended Data Fig. 6c, p=0.01).

The landscape of copy-number variations, assessed on the TCGA SARC cohort, revealed differences between histologies, consistent with previously been described observations[8]. However, no notable CNV difference was found between SICs (data not shown).

***In situ* validation of SIC profiles in tumours**

To validate *in situ* the TME profiles of SICs, we analysed an independent cohort of 93 STS cases (NTUH cohort, Extended Data Table 1). 73 samples passed quality control for transcriptomic analysis using Nanostring nCounter technology. We classified this cohort into the same 5 SICs (see methods) with the following distribution: A: 16 (21.9%), B: 19 (26.0%), C: 10 (13.7%), D: 17 (23.3%), E: 11 (15.1%). The NTUH cohort samples exhibited gene-

147 expression-based TME profiles similar to that of TCGA SARC and GSE21050 cohorts
148 (Extended Data Fig. 7a).

149 By quantitative immunohistochemistry (IHC), “Immune desert” SIC A was characterized by
150 very low densities of $CD3^+$, $CD8^+$ or $CD20^+$ cells, whereas “Immune and TLS high” SIC E
151 exhibited high densities of these cells (pairwise comparison, p=4.01e-06, p=6.64e-06 and
152 p=9.90e-07, respectively). The “vascularised” SIC C exhibited a moderate infiltration by
153 immune cells and high density of $CD34^+$ endothelial cells (Extended Data Fig. 7b-c).

154 **Tertiary lymphoid structures are a feature of SIC E tumours**

155 The B cell-specific chemotactic cytokine CXCL13, which is associated with the presence of
156 TLS[12], was strongly expressed in SIC E tumours (Fig. 1c, Extended Data Fig. 2c). Its expression
157 highly correlated with that of the TLS-associated 12-chemokine signature[13] (Extended Data
158 Fig. 8a), suggesting that TLS could be a marker of SIC E. TLS were defined as a $CD20^+$ B cells
159 follicle juxtaposed to a $CD3^+$ T cells aggregate containing at least one DC-$Lamp^+$ mature
160 dendritic cell[12,14–16] (Fig. 3a, left). A strong association between SICs and the presence of TLS
161 was identified (p=3.13e-06, Fig. 3c). No TLS were observed in tumours from SICs A, C and D,
162 and only one tumour from SIC B had one TLS. Conversely, nine out of eleven (82%) SIC E
163 tumours exhibited one or more TLS. All TLS were intratumoural (Extended Data Fig. 8b), and
164 found at the periphery and in the centre of the tumour in all histologies (Extended Data Fig. 8c-
165 d).

166 We observed the presence of $CD3^+$PD-1^+ T cells (Fig. 3a, right) in the germinal centre (GC) of
167 TLS with characteristics of follicular helper T cells (positive for CD4, PD-1 and the CXCL13
168 receptor CXCR5, Fig. 3b, left)[17,18], $CD23^+CD21^+$ cells with reticular morphology characteristic
169 of follicular dendritic cells and $PNAd^+$ structures with high endothelial venules morphology
170 (Fig. 3b, right). GC are a hallmark of secondary follicle-like TLS (SFL-TLS), the final

maturation step of TLS, the earlier steps being early TLS (E-TLS), and primary follicle-like TLS (PFL-TLS)[15,16]. E-TLS, PFL-TLS and SFL-TLS represented respectively 60.5%, 21.1% and 18.3% of all TLS analysed (Extended Data Fig. 8e-f). This differed between histologies (p=7.76e-05), with UPS having only 16.7% of E-TLS.

Tumours with TLS (11.8%, 11/93) had significantly higher densities of tumour-infiltrating $CD3^+$ T cells (p=4.0e-05), $CD8^+$ T cells (p=1.8e-04) and $CD20^+$ B cells (p=1.5e-05) (Fig. 3d). This association persisted even if T and B cells within TLS were excluded from the analysis (p=1.5e-04, p=3.8e-04 and p=7.9e-07, respectively, Fig. 3d), reflecting that high immune cell infiltration is not limited to TLS.

SICs predict response to PD-1 blockade in STS patients

We examined if SICs can predict patient response to checkpoint blockade therapy. We obtained 47 pre-treatment STS metastasis biopsies from patients enrolled in the SARC028 clinical trial[4] and its expansion cohort[10] (Extended Data Table 1), which evaluated the efficacy of pembrolizumab (an anti-PD-1 monoclonal antibody) in patients with metastatic STS. Of these 47 patients, 1 achieved a complete response (CR), 9 partial response (PR), 17 stable disease (SD) and 20 had progressive disease (PD) (Fig. 4a). Pre-treatment tumours were classified into SICs based on gene expression data. The Objective Response Rate (ORR, accounting for complete and partial responses) as evaluated by RECIST criteria was 21.2 % in the overall cohort. SICs however showed substantial variation in ORR, with SIC E patients exhibiting the highest ORR (50%, 5/10), followed by SIC D (25%, 3/12) and SIC C (22%, 2/9) (Fig. 4a). CR were only found in SIC E, as well as one patient who had 100% change in target lesions but non-CR in non-target lesions and thus not qualifying for CR. Strikingly, there were no responders within the SIC A (0/5) and B (0/11) groups (Fig. 4a). Overall, SIC E tumours were associated with the highest response rate to pembrolizumab in comparison with tumours from other SICs (p=0.026, Fig. 4b). Patients with SIC E tumours also exhibited improved

progression-free survival than patients with SIC A or B tumours (p=0.023 and p=0.0069, respectively, Fig. 4c).

DISCUSSION AND CONCLUSION

This study is the most comprehensive analysis of STS immune tumour microenvironment and the first to evaluate the prognostic impact of immune infiltrates by simultaneously integrating multiple immune cell populations and malignant cell characteristics. There have been prior efforts to examine the immune profile of STS tumours, although the significance of the B cells and TLS were not investigated. The clinical impact of $CD8^+$ T cells and PD-1 expression has yielded controversial results[7,8,19–25]. Here, we found $CD8^+$ T cells signature and PD-1 to be expressed in D and E SICs, which are associated with favourable outcomes, providing high B cell infiltrate. The present integrative analysis demonstrates that infiltration by B cells is a key discriminative feature of a group of patients with improved survival. This B cell-high group was, in particular, found to respond better to PD-1 blockade therapy, although this should be validated on a larger cohort.

The field of immuno-oncology is rapidly expanding and it is critical to accurately identify patients likely to respond. Here, we propose a new classification for STS that is immune-centric with prognostic impact. It defines a group with better response to anti-PD-1 therapy marked by B cells and TLS. This finding may have broad applications. Indeed, sarcomas are considered as immune quiescent tumours, with a low mutational burden. Nevertheless, our data show that some STS are immunogenic and that this is driven by B cells. Further work is needed to extend these findings to all STS histologies and other cancers. Similarly, the underlying mechanisms require further investigation, but a possible explanation is that TLS are sites where anti-tumoral immunity is generated with B cells instructing T cells, in particular $CD8^+$ T cells, to recognize tumour-associated antigens[26]. It is indeed striking that TLS-rich tumours are more infiltrated by $CD8^+$ T cells. These T cells can become exhausted, explaining the correlation of expression of

immune checkpoints (PD-1, Lag-3, …) with TLS, and why treatment with checkpoint inhibitors may allow productive anti-tumour immunity in TLS-rich tumours. Overall, our findings lay the foundation for a tool to risk-stratify STS patients and identify those who may be more likely to benefit from immunotherapies, and may be broadly applicable to other malignancies[26–30].

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Figure legends

Figure 1: The Sarcoma Immune Classes exhibit strongly different tumour microenvironments.

This figure refers to the TCGA SARC cohort (n=213). **a** Composition of the TCGA SARC cohort by SIC, and histology. **b** Composition of the TME by sarcoma immune class (SIC) as defined by the MCP-counter Z-scores. **c** Expression of gene signatures related to the functional orientation of the immune TME by SIC. **d** Expression of genes related to immune checkpoints by SIC. Adjusted p-values are obtained from Benjamini-Hochberg correction of two-sided Kruskal-Wallis tests p-values.

Figure 2: SICs and B cells are predictive of STS patients' survival

This figure refers to TCGA SARC and GSE21050 pooled cohorts (n=496). **a** Overall survival of STS patients by SIC of their tumour. **b** Multivariate Cox proportional regression outcome, with all included variables represented. For each variable, the reference level is the first one. A grey bar indicates a p-value above 0.05, and variables indicated by green and red bars significantly associated with prognosis in this multivariate model, respectively positively and negatively. Error bars represent 95% confidence interval. **c-d** Overall survival of STS patients according to MCP-counter scores for $CD8^+$ T cells (**c**) or B lineage (**d**). **e** Overall survival of patients based on the tumoural infiltration by B lineage cells and $CD8^+$ T cells. The analyses were performed with the Kaplan-Meier estimates and two-sided logrank tests. Tumours were considered high for $CD8^+$ T cells if their score was above median, and for cytotoxic lymphocytes and B lineage if their score was above the third quartile, in each cohort. FNCLCC: Fédération Nationale des Centres de Lutte Contre le Cancer.

Figure 3: Tertiary lymphoid structures (TLS) are a distinguishing feature of the immune-high class of STS.

This figure refers to the NTUH cohort (n=93). **a** Populational characterization of tertiary lymphoid structures. Left, examples of two tertiary lymphoid structures by IHC, identified as $CD3^+$ T cell (blue) aggregates containing DC-$Lamp^+$ mature dendritic cells (red, red arrows) and juxtaposing $CD20^+$ B cell aggregates (brown). Right, representative immunofluorescence staining of a TLS for CD3 (magenta), CD20 (green) and PD-1 (cyan). DAPI staining is shown in blue. The multispectral image shows $CD3^+PD\text{-}1^+$ double positive cells (yellow arrows). **b** Functionality of tertiary lymphoid structures. Left, $CXCR5^+$ (magenta), $CD4^+$ (yellow) and $PD\text{-}1^+$ (green) cells in zones 3 and 4 of the same TLS. Multispectral fluorescence images of zones 3 and 4 show $CXCR5^+CD4^+PD\text{-}1^+$ triple positive cells (red arrows) characteristic of T follicular helper cells. Right, $CD20^+$ cells stained in pink (left) on consecutive sections of a TLS. CD23 (green on left) and CD21 (brown on right) positive cells with reticular morphology characteristic of follicular dendritic cells (yellow arrow, zone 1). $PNAd^+$ structures (brown, green arrow) with high endothelial venule morphology are also detectable nearby (zone 2). **c** Number of TLS among 5 SICs of 73 tumours of NTUH cohort (n=73). **d** Characterization of the immune infiltrate in tumours according to TLS presence (TLS^- n=82, TLS^+ n=11, total n=93). Densities of $CD3^+$ (left), $CD8^+$ (centre) and $CD20^+$ (right) cells in tumours lacking TLS (TLS^-) or containing TLS (TLS^+); densities including (total) or excluding (excl) TLS are indicated for the TLS^+ tumours. Boxplots represent median (larger bar) and interquartile range (IQR). Upper whisker extends to whichever is minimal, maximum or 3^{rd} quartile plus 1.5*IQR. Lower whisker extends to whichever is maximal, minimum or first quartile minus 1.5*IQR. For panel **d**, p-values were computed with two-sided Mann-Whitney tests.

Figure 4: SICs are strongly associated with STS response to PD-1 blockade therapy.

This figure refers to the SARC028 cohort (n=47). **a** Relationship between SIC, histology and response to treatment in the SARC028 cohort. **b** Waterfall plot showing the best response to pembrolizumab as percentage change in size of target lesions from baseline (n=45). Tumour sizes were calculated as the sum of target lesion diameters. The colours indicate the SIC to which each tumour was assigned. Dashed lines indicate +20%, -30% and -100% change from baseline levels. SIC E vs other comparison was performed using a two-sided Mann-Whitney test. **c** Progression-free survival of patients by tumour SIC (n=47). DDLPS: dedifferentiated liposarcoma, LMS: leiomyosarcoma, UPS: undifferentiated pleomorphic sarcoma, SS: synovial sarcoma.

METHODS (2039 words)

Ethics and patients

Patients diagnosed with dedifferentiated liposarcoma, leiomyosarcoma, and undifferentiated pleomorphic sarcoma were identified and the pathology diagnosis confirmed by a certified pathologist in National Taiwan University Hospital. The research was approved by the Research Ethics Committee of NTUH (201605061RINA). Informed consent was obtained from all subjects. Formalin-fixed paraffin embedded (FFPE) blocks were retrieved and 4-5 μm thickness slides were taken for immunohistochemistry staining and RNA extraction for Nanostring testing. Other cohorts were previously published[4,8,31–35].

Establishing the immune classification of STS

To establish a robust immune classification of STS, publicly available transcriptomic data from The Cancer Genome Atlas (TCGA) data portal and Gene Expression Omnibus (GEO) repository representing 4 large and independent patient cohorts were included. Only tumours from the most common histologies of genomically complex STS were included: leiomyosarcoma (LMS), undifferentiated pleomorphic sarcoma (UPS) and dedifferentiated liposarcoma (DDLPS). We analysed data from the TCGA SARC[8] (n = 213), GSE21050[31] (n = 283), GSE21122[32] (n = 72) and GSE30929[33] (n = 40) cohorts.

Public transcriptomic data pre-processing

Transcriptomic data was downloaded from the TCGA data portal (SARC cohort) and Gene Expression Omnibus (Accession codes: GSE21050, GSE21122 and GSE30929). TCGA SARC was restricted to complex genomics sarcomas (UPS, DDLPS and LMS). Normalized TCGA SARC RNA-Seq data was log2-transformed. Micro-array data was normalized using frozen-RMA method[36] from the R package frma. Batch effect was corrected across series using ComBat[37], with histology as covariate.

Estimation of the TME composition

TME composition of each tumour was assessed with the MCP-counter tool[9], which provides abundance scores for 8 immune (T cells, $CD8^+$ T cells, cytotoxic lymphocytes, NK cells, B cell lineage, monocytic lineage, myeloid dendritic cells and neutrophils), and 2 stromal populations (endothelial cells and fibroblasts). The scores are based on analysis of transcriptomic markers, i.e. transcriptomic features that are strongly, specifically and stably expressed in a unique cell population. These scores are proportional to each cell population's abundance in the tumour, therefore allowing inter-sample comparison and large cohort analyses[38]. The MCP-counter signatures composition are as follows: T cells: *CD28, CD3D, CD3G, CD5, CD6, CHRM3-AS2, CTLA4, FLT3LG, ICOS, MAL, MGC40069, PBX4, SIRPG, THEMIS, TNFRSF25, TRAT1*; $CD8^+$ T cells: *CD8B*, cytotoxic lymphocytes: *CD8A, EOMES, FGFBP2, GNLY, KLRC3, KLRC4, KLRD1*; B lineage: *BANK1, CD19, CD22, CD79A, CR2, FCRL2, IGKC, MS4A1, PAX5;* NK cells: *CD160, KIR2DL1, KIR2DL3, KIR2DL4, KIR3DL1, KIR3DS1, NCR1, PTGDR, SH2D1B*; monocytic lineage: *ADAP2, CSF1R, FPR3, KYNU, PLA2G7, RASSF4, TFEC*; myeloid dendritic cells: *CD1A, CD1B, CD1E, CLEC10A, CLIC2, WFDC21P*; neutrophils: *CA4, CEACAM3, CXCR1, CXCR2, CYP4F3, FCGR3B, HAL, KCNJ15, MEGF9, SLC25A37, STEAP4, TECPR2, TLE3, TNFRSF10C, VNN3*; endothelial cells: *ACVRL1, APLN, BCL6B, BMP6, BMX, CDH5, CLEC14A, CXorf36, EDN1, ELTD1, EMCN, ESAM, ESM1, FAM124B, HECW2, HHIP, KDR, MMRN1, MMRN2, MYCT1, PALMD, PEAR1, PGF, PLXNA2, PTPRB, ROBO4, SDPR, SHANK3, SHE, TEK, TIE1, VEPH1, VWF*.

Intra-cohort immune classifications

Fibroblasts signature was removed from this analysis as all STS tumours exhibited high and homogeneous scores for this cell population, which is consistent with the mesenchymal origin of STS. The signature for CD8 T cells was removed from the analysis for GSE21050, GSE21122 and GSE30929 as it showed very small variation across all samples in these

microarray-based cohorts. Unsupervised clustering of samples in each cohort was performed based on the metagene Z-score for the included populations of MCP-counter (Extended Data Fig. 9a-d) using R software, with the Euclidian distance and Ward's linkage criterion, using the gplots package. The TCGA SARC, GSE21050, GSE21122 and GSE30929 cohorts were respectively separated into 6, 9, 7 and 6 groups. The number of clusters was chosen empirically following the dendrograms shown in Extended data Fig. 9a-d. Analysis of the inter-sample variance revealed that much of the explainable variance was already attained at the chosen number of clusters as visualized in Extended Data Fig. 9e-h.

Pan-cohort immune classes

To aggregate the above four intra-cohort classifications, the transcriptome matrix of each cohort was independently zero-centred for each gene across all samples. Then we computed the centroids of each class over the whole transcriptome and analysed the Pearson correlations between all the centroids on the set of genes shared across the four cohorts (Extended Data Fig. 9i). From these correlations, we deduced 5 Sarcoma Immune Classes (SICs). The tumours from 6 remaining cohort-specific clusters shared intermediate/weak correlation patterns to other clusters and were temporarily labelled as "Unclassified".

Prediction of the immune classes

Centroids of SICs were computed on MCP-counter intra-series Z-scores for T cells, cytotoxic lymphocytes, B cell lineage, NK cells, monocytic lineage, myeloid dendritic cells, neutrophils and endothelial cells, on all cohorts. To predict *de novo* the immune classes of each of the cohorts, MCP-counter Z-scores were computed, and each sample was assigned to the closest immune class based on its Euclidian distance to the related centroids. The SICs labels used in the article are the ones predicted using this method. Principal component analysis of the 608 samples on the MCP-counter scores shows that the intra-SIC homogeneity was improved by

this prediction step (Extended Data Fig. 9j-k), as confirmed by supervised tests across SICs (Extended Data Fig. 9l-m).

Gene signatures for the functional orientation

The signatures used to determine the functional orientation of the TME were derived from the literature[39]. The signatures were the following: Immunosuppression (*CXCL12, TGFB1, TGFB3, LGALS1*), T cell activation (*CXCL9, CXCL10, CXCL16, IFNG, IL15*), T cell survival (*CD70, CD27*), regulatory T cells (*FOXP3, TNFRSF18*), major histocompatibility complex I *(HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, HLA-G, B2M*), myeloid cell chemotaxis (*CCL2*), and tertiary lymphoid structures (*CXCL13*). For each signature, scores were computed as the geometric mean signature expression.

De novo prediction of the SICs of additional cohorts and other platforms

Prediction of the immune classes on new samples

The predictor described above was adapted to analyse new and independent samples, from Nanostring-analysed FFPE samples. In a first step, SICs were estimated on the NTUH cohort by sorting samples on the B lineage signature, T cells signature then Endothelial cell signature and assigning each sample according to the SIC it resembled the most. Similarly to what was done above, centroids of each SIC on Nanostring data MCP-counter scores Z-scores were computed and samples were reassigned to the SIC they were closest to the centroid of. For new samples from the SARC028 cohort, MCP-counter scores for T cells, Cytotoxic lymphocytes, B lineage and Endothelial cells were computed and transformed as Z-scores. Distances with Nanostring-defined centroids presented above were computed with Euclidian metric, and samples were assigned to the SIC with the lowest distance.

RNA extraction from FFPE tumours

Human FFPE tumour specimens were cut into 3 μm thick sections and were reviewed under microscope for tumour histology. Non-tumour tissues were excluded and tumour tissues were deparaffinized by deparaffinization solution (Qiagen cat. # 19093) and RNA were extracted by RNeasy FFPE kit (Qiagen cat. # 73504) according to the manufacturer's protocol. RNA quality and size distribution was determined by the Agilent 2100 Bioanalyzer with RNA analysis kits (RNA 6000 nano kit cat. # 5067-1511, RNA 6000 nano reagent cat. # 5067-1512, RNA 6000 nano ladder cat. # 5067-1529, RNA 6000 pico kit cat. # 5067-1513, RNA 6000 pico reagents cat. # 5067-1514, RNA 6000 pico ladder cat. # 5067-1535) for cohorts NTUH core and NTUH whole, and by the Agilent RNA ScreenTape assay (catalogue # : RNA ScreenTape 5067-5576, RNA ScreenTape sample buffer 5067-5577, RNA ScreenTape ladder 5067-5578) and Agilent 2200 TapeStation for cohort SARC028. The samples from SAR028 were separately quality controlled by the sarcoma pathology group at MD Anderson Cancer Center.

Nanostring nCounter analysis

The RNA was analysed using the nCounter Technology (Nanostring Technologies) as per the manufacturer's protocol. Data were normalized using the nSolver software (Nanostring Technologies).

Enzymatic and fluorescent multiplexed immunohistochemistry

The FFPE human tumour and control specimens were cut into 3-μm-thick sections. Human FFPE tonsil sections were used as positive controls for CD3, CD4, CD8, CD20, CD21, CD23, CD34, CXCR5, DC-Lamp, PD1, PD-L1 and PNAd, placenta sections were used in addition for PD-L1 and cerebral cortex tissue was used as a negative control. The specificity of all the antibodies were tested by the manufacturers and the specificity of anti-PD-1 antibodies was validated in our laboratory on overexpressing cells pellets as previously reported[40]. Antigen retrieval was carried out on a PT-link (Dako) using the EnVision FLEX Target Retrieval

Solutions at High pH (Dako, K8004) or Low pH (Dako, K8005). Endogenous peroxidase activity and non-specific Fc receptor binding were blocked with H2O2 3% (Gifrer, 10603051) and Protein Block (Dako, X0909) respectively. The primary and secondary antibodies used for IHC and IF are summarized in Extended data table 2. IHC and IF images were independently analysed by three observers (LL, CSF, GL).

Enzymatic immunohistochemistry

The stainings were performed with an Autostainer Link 48 (Dako). Chromogenic detection was performed using 3,3'-diaminobenzidine (Dako, K3468) for CD8, CD20, CD21, PD-L1 and PNAd, 3-amino-9-ethylcarbazole substrate (Vector Laboratories, SK-4200) for DC-Lamp, Blue Alkaline Phosphatase Substrate (Vector Laboratories, SK5300) for CD3, HighDef red IHC chromogen (AP) (Enzo, ADI-950-140-0030) for CD20 and Permanent HRP Green (Zytomed Systems, ZUC070-100) for CD23 and CD34. The nuclei were counterstained with haematoxylin (Dako, S3301). After mounting with Glycergel Mounting Medium (Dako, C056330-2) or EcoMount (Biocare Medical, EM897L), the slides were scanned with a Nanozoomer (Hamamatsu). For CD3, CD8, CD20 and DC-Lamp markers, the density of positive cells/mm² was quantified with Calopix Software (Tribvn). For CD34 marker, the density of positive vessels/mm² was quantified with Halo10 software (Indica labs). TLS were identified using the registration module to fit one slide on the other (Halo10 software, Indica labs). Tumours were considered TLS-positive when a CD3 aggregate with DC-Lamp staining was found juxtaposing a CD20 aggregate. Only aggregates with surface above 60,000 µm², containing at least 700 cells and at least 350 $CD20^+$ cells were considered.

Fluorescent multiplexed immunohistochemistry

For the PD1/CD20/CD3 3-plex staining, a tyramide system amplification (TSA) was used. The stainings were performed with a Leica Bond RX. The incubation with TSA reagent was

performed after the incubation of the HRP-conjugated polymer and was followed by antibody stripping at 97°C for 10 minutes. This protocol was repeated for the second and third primary antibodies and corresponding polymer incubations. The dilutions used for the TSA are 1:400 for TSA AF488, 1:800 for TSA AF594 and 1:200 for TSA AF647 following the manufacturer's recommendations. For the CXCR5/CD4/PD-1 3-plex staining, we used a conventional fluorescent-dye conjugated secondary antibody system performed manually (all secondary antibodies were diluted at 1:100). For all the fluorescent stainings, the nuclei were stained with DAPI Solution (Thermo Fisher, 62248) at 2µg/ml for 10 minutes. After mounting with ProLongTM Gold Antifade Mountant (Thermofisher, P36934), the slides were scanned with a Zeiss Axio Scan.Z1.

Statistical analysis

All statistical analyses were performed using the R software (version 3.4.4) and the packages survival, gplots, dunn.test and FactoMineR. The relationship between two categorical variables was estimated with the chi-square test. The relationship between a categorical variable and a quantitative variable was estimated with the Mann-Whitney U test (2 categories) or the Kruskall-Wallis test (3 or more categories). All tests were two-sided. In cases with 3 or more categories, pairwise comparisons were carried with Dunn tests. The relationship between two quantitative variables was estimated with the Pearson correlation. When appropriate, p-values were corrected for multiple hypothesis testing with the Bonferroni or Benjamini-Hochberg methods, as specified in the text or figure legends. Survival was analysed with Kaplan-Meier estimates and logrank tests. Box plots represent median (larger bar) and interquartile range (IQR). Upper whisker extends to whichever is minimal, the maximum of the data or the 3rd quartile plus 1.5*IQR. Lower whisker extends to whichever is maximal, the minimum of the data or the first quartile minus 1.5*IQR.

Data and Code Availability statement

The transcriptomic datasets analysed in this study can be accessed on the GDC Portal (portal.gdc.cancer.gov, cohort TCGA SARC) and the Gene Expression Omnibus repository (www.ncbi.nlm.nih.gov/geo, accession numbers GSE21050, GSE21122, GSE30929). FSG cohort data is publicly available from ArrayExpress for GIST (www.ebi.ac.uk/arrayexpress, accession code E-MTAB-373) and from Gene Expression Omnibus for synovial sarcomas (www.ncbi.nlm.nih.gov/geo, accession number GSE40021). Myxoid liposarcomas from the FSG cohort are available upon reasonable request to the authors. Immunohistochemistry and gene expression data related to NTUH cohort (Fig. 3, Extended Data Figs. 7 and 8) are available upon reasonable request to WHF (herve.fridman@crc.jussieu.fr). The data that support the findings related to Fig. 4 are available from SARC but restrictions apply to the availability of these data, which were used under license for the study. Data are however available upon reasonable request to HAT (HTawbi@mdanderson.org) and with permission of SARC. All code used in this study is available from the authors upon reasonable request.

Additional references for methods

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Author contributions

FP, WHF, CSF, AdR, TWC, HAT, and AI designed the study and experiments. FP, AdR, CL and YL performed the bioinformatics analysis. LL, GL, IN, LPH, AB, MM and FP carried the immunohistochemistry experiments. JC, YMJ and JA performed anatomo-pathology revision on the samples. EZK, CMS, WLW and KMW performed the RNA extraction and nanostring experiments. TWC, AI, MT and HAT provided clinical guidance. TWC, MT, AI, EZK, AJL, CLR, MAB, VB, DR and HAT cared for the patients and provided patient materials or clinical data. FP, WHF, CSF, HAT, AdR, EZK, CLR, AJL, TWC, CMS, JAW and AI discussed the data and wrote the text. WHF, CSF, AdR and HAT supervised the study and all authors commented on the manuscript and approved the submission.

Competing interest declaration

WHF is a consultant for AstraZeneca, Novartis, Servier and Pierre Fabre. AI serves in the advisory board of Bayer, Daiichy, Epizyme, Lilly, Novartis, Roche and Springworks, and received research funding from Astra Zeneca, Bayer, Chugai, Merck, MSD, Novartis and Pharmamar. JA is a consultant for AstraZeneca, Bayer, BMS, MSD and Roche and received research funding from MSD, Pfizer and Pierre Fabre. JAW participated on advisory boards for Merck, BMS, Novartis, Astra Zeneca, Roche Genentech and Illumina. MAB is a consultant for EMD Serono, Immune Design, Eisai. HAT serves on advisory boards and receives consulting fees from BMS, Merck and Genentech, and received research funding from BMS, Merck, Celgene, GSK, and Genentech. TWC participated in advisory boards for Eisai and Lilly and received research funds from Eisai. CLR received research funding from BMS. The other authors declare no conflict of interest.

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Extended Data Figure Legends

Extended Data Fig. 1: Diagram representation of analytic workflow

Extended Data Fig. 2: SICs in various STS histologies.

a Repartition of the SICs in various histologies of TCGA SARC and GSE21050 (leiomyosarcoma [LMS], undifferentiated pleomorphic sarcoma [UPS], dedifferentiated liposarcoma [DDLPS]), and FSG cohort (synovial sarcoma, myxoid liposarcoma, gastrointestinal stromal tumour [GIST]).

b Survival of patients from the FSG cohort (n=136) according to SIC classification. Patients with synovial sarcoma, myxoid liposarcoma and GIST were pooled. The analysis was performed with the Kaplan-Meier estimates and two-sided logrank tests.

Extended Data Fig. 3: The Sarcoma Immune Classes exhibit strongly different tumour microenvironments.

This figure refers to the GSE21050 cohort (n=283). **a** Composition of the GSE21050 cohort by SIC, histology and site of disease. **b** Composition of the TME by sarcoma immune class (SIC) as defined by the MCP-counter Z-scores. **c** Expression of gene signatures related to the functional orientation of the immune TME by SIC. **d** Expression of genes related to immune checkpoints by SIC. Adjusted p-values are obtained from Benjamini-Hochberg correction of two-sided Kruskal-Wallis tests p-values. These observations stand for cohorts GSE21122 and GSE30929 (not shown).

Extended Data Fig. 4: Distribution of MCP-counter scores in TCGA SARC (n=213) (**a**,**c**,**e**) and GSE21050 (n=283) (**b**,**d**,**e**), for $CD8^+$ T cells (**a**,**b**), cytotoxic lymphocytes (**c**,**d**) and B lineage (**e**,**f**). The blue line indicates the density curve. The red dotted line indicates the cut-off chosen to segregate high or low values, set at the median for $CD8^+$ T cells and at the third quartile for cytotoxic lymphocytes and B lineage, in each cohort. These values were chosen since the CD8 T cells scores present a normal distribution, while the cytotoxic lymphocytes and B lineage scores distribution exhibit a long right tail.

Extended Data Fig. 5: B cell infiltration of STS is the key factor associated with overall survival

This figure refers to TCGA SARC and GSE21050 pooled cohorts (n=496). **a** Overall survival of STS patients according to MCP-counter scores for cytotoxic lymphocytes. **b** Overall survival of patients based on the infiltration level of their tumours by B lineage cells and cytotoxic lymphocytes. **c-e** Overall survival of patients based on degree of tumour infiltration by B lineage cells and expression of (**c**) *PDCD1*, (**d**) *CD274* and (**e**) *FOXP3*. The analyses were performed with the Kaplan-Meier estimates and two-sided log-rank tests. Tumours were considered high for expression of *PDCD1*, *CD274* and *FOXP3* if their expression was above median, and high for B lineage and cytotoxic lymphocytes if the MCP-counter score was above the third quartile.

Extended Data Fig. 6: The mutational landscape of STS tumours does not vary significantly between SICs.

This figure refers to the TCGA SARC cohort (n=213). **a** Mutational burden according to the SIC of the tumours, expressed in number of non-silent mutations. P-value was computed with

a Kruskal-Wallis test. Boxplots represent median (larger bar) and interquartile range (IQR). Upper whisker extends to whichever is minimal, maximum or 3rd quartile plus 1.5*IQR. Lower whisker extends to whichever is maximal, minimum or first quartile minus 1.5*IQR. **b** Mutation frequency of all genes that are mutated in greater than 2.5% of tumours. **c** Mutation frequency for genes that are mutated in more than 5% of tumours, according to SICs in the TCGA SARC cohort. The dashed lines indicate the overall mutation frequency. P-values were obtained through one-sample two-sided t-tests, corrected for multiple testing with the Bonferroni method. This was applied only to samples which had mutations on the considered genes (TP53: n=75; ATRX: n=34; TTN: n=21; RB1: n=19; MUC16, n=17; PCLO, n=13; DNAH5, MUC17 and USH2A: n=11, PTEN, n=6; KRAS, n=2; BRAF, n=1).

Extended Data Figure 7: Validation of SIC profiles by immunohistochemistry.

This figure refers to the NTUH cohort **a** SIC attribution as defined by gene expression using the MCP-counter Z-scores in 73 cases **b** Cell density counts showing the differences in TME composition according to SIC identification of the 73 cases (SIC A: n=16; SIC C: n=10; SIC E: n=11). P-values labelled KW are derived from two-sided Kruskal-Wallis tests. Pairwise comparisons are derived from the Dunn test. Boxplots represent median (larger bar) and interquartile range (IQR). Upper whisker extends to whichever is minimal, maximum or 3rd quartile plus 1.5*IQR. Lower whisker extends to whichever is maximal, minimum or first quartile minus 1.5*IQR. **c** Representative images of CD3 (green)/CD20 (pink), CD8 (brown) and CD34 (green) expression by immunohistochemistry of SIC A, C and E tumours. The same area of the tumour is represented (0.05 mm^2) in each image. Similar results were observed on the other tumours from the same SICs (SIC A: n=16; SIC C: n=10; SIC E: n=11).

Extended Data Fig. 8: Location and maturation of TLS

a Pearson correlation between the expression of CXCL13 and the 12-chemokine signature of TLS in TCGA SARC cohort (n=213). Samples are coloured according to SICs. **b** Intratumoural location of TLS in three different examples from the NTUH cohort, respectively DDLPS, UPS and LMS. TLS are observed by the presence of $CD20^+$ B cells aggregates (brown, surrounded by blue shapes). The red line delineates the tumoural zone. Similar findings were observed on the 11 tumours with TLS. **c** Definition of peripheral, medium and central zones, accounting for 25%, 25% and 50% of the total tumour area, respectively. **d** Distribution of TLS in the various zones. Each bar represents one tumour. The letters above bars indicates the SIC of the tumour when the sample passed quality control of Nanostring nCounter hybridization. Dots indicate tumours in which SIC could not be determined because of RNA quality control. Similar images were observed over 66 E-TLS, 23 PFL-TLS and 20 SFL-TLS. **e** Illustration of diverse degrees of TLS maturation in STS tumours. Consistent with maturation events occurring in secondary lymphoid organs, three maturation steps have been described for TLS: early follicle (E-TLS, bottom), primary follicle-like (PFL-TLS, middle) and secondary follicle-like with germinal centre (SFL-TLS, top) which differ in the presence of follicular dendritic cells (FDC) and in their markers. E-TLS contain aggregates of $CD20^+$ B cells and $CD3^+$ T cells without FDC, PFL-TLS contain $CD21^+$ FDC (red dotted zones) and SFL-TLS contain a germinal centre, notably visible through the presence of CD21+CD23+ FDC (yellow dotted zone). DAPI staining is shown in white. DAPI-negative green dots correspond to fluorescent erythrocytes. **f** Distribution of TLS maturation steps in a subset of tumours. Each bar represents one tumour. Differences between the number of TLS observed here and in other figures can be explained by use of non-consecutive slides or a different tumour block for some samples.

Extended Data Fig. 9: Pan-cohort immune classification

This figure refers to the four discovery cohorts: TCGA SARC (n=213), GSE21050 (n=283), GSE21122 (n=72) and GSE30929 (n=40). **a-d** Heatmap and unsupervised hierarchical clustering of the MCP-counter scores describing the tumour microenvironment. Each of the population is represented by the Z-scores of the signature. **a** TCGA SARC. **b** GSE21050. **c** GSE21122. **d** GSE30929. **e-h** Evolution of the variance explained by the clusters as a function of the number of clusters. The red dots indicate the number of clusters that was retained in this study. Each graph corresponds to the heatmap that is situated on its left. **i** Heatmap of the Pearson correlation of centroids from each SIC class of discovery cohorts (TCGA SARC, GSE21050, GSE21122, GSE30929, n=608), with 5 immune classes and 2 groups of unclassified samples. **j-k** Principal component analysis of samples from the four discovery cohorts (n=608), based on their normalized and merged MCP-counter scores. (**j** is coloured according to the original classes, **k** is coloured according to the predicted immune classes, showing a heightened homogeneity within each SIC class). **l-m** Composition of the TME with classes defined as in **j** and **k** for the four discovery cohorts (n=608), expressed in cohort-specific row Z-scores.

Extended Data Tables titles and footnotes

Extended Data Table 1: Clinicopathological composition of the cohorts included in this study. For cohort GSE21050, sex information could not be retrieved for 14 patients. For cohort NTUH, SIC could be determined for 73 patients only. NA: Not Available

Extended Data Table 2: Antibodies used for immunohistochemistry and immunofluorescence

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to

F. Petitprez, A. de Reyniès, E. Z. Keung, T.W. Chen, C.M. Sun, J. Calderaro, Y.M. Jeng, L.P. Hsiao, L. Lacroix, A. Bougoüin, M. Moreira, G. Lacroix, I. Natario, J. Adam, C. Lucchesi, Y. Laizet, M. Toulmonde, M. A. Burgess, V. Bolejack, D. Reinke, K. M. Wani, W.L. Wang, A. J. Lazar, C. L. Roland, J. A. Wargo, A. Italiano , C. Sautès-Fridman, H. A. Tawbi and W.H. Fridman

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I agree with these changes.

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Pour : Florent Petitprez <Florent.Petitprez@ligue-cancer.net>, Keung,Emily <ekeung@mdanderson.org>, Wei-Wu (Tom) Chen <saxotomy@gmail.com>, Cheng-Ming Sun <cheng-ming.sun@crc.jussieu.fr>, Julien Calderaro <julien.calderaro@hmn.aphp.fr>, liippiil@yahoo.com.tw <liippiil@yahoo.com.tw>, mRNA0912@yahoo.com.tw <mRNA0912@yahoo.com.tw>, laetitia.lacroix@inserm.fr <laetitia.lacroix@inserm.fr>, antoine.bougouin@crc.jussieu.fr <antoine.bougouin@crc.jussieu.fr>, guillaume.lacroix@crc.jussieu.fr <guillaume.lacroix@crc.jussieu.fr>, Ivo Gameiro Natario <ivo.natario@gmail.com>, ADAM Julien <julien.adam@gustaveroussy.fr>, Lucchesi Carlo <c.lucchesi@bordeaux.unicancer.fr>, Laizet Yechan <y.laizet@bordeaux.unicancer.fr>, Toulmonde Maud <m.toulmonde@bordeaux.unicancer.fr>, Burgess, Melissa <burgessma@upmc.edu>, vanessab@crab.org <vanessab@crab.org>, Reinke, Denise <DReinke@sarctrials.org>, Kwani@mdanderson.org <Kwani@mdanderson.org>, Lazar,Alexander <alazar@mdanderson.org>, Roland,Christina Lynn <CLRoland@mdanderson.org>, Wargo,Jennifer <JWargo@mdanderson.org>, Italiano Antoine <A.Italiano@bordeaux.unicancer.fr>, catherine fridman <catherine.fridman@crc.jussieu.fr>, Tawbi,Hussein <HTawbi@mdanderson.org>, FRIDMAN Hervé <herve.fridman@crc.jussieu.fr>, marco moreira <Marco.moreira@outlook.fr>, wlwang@mdanderson.org <wlwang@mdanderson.org>

Dear Florent,

The list of authors of manuscript 2018-06-08855D, titled "B cells are associated with sarcoma survival and immunotherapy response", was changed from

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to

F. Petitprez, A. de Reyniès, E. Z. Keung, T.W. Chen, C.M. Sun, J. Calderaro, Y.M. Jeng, L.P. Hsiao, L. Lacroix, A. Bougoüin, M. Moreira, G. Lacroix, I. Natario, J. Adam, C. Lucchesi, Y. Laizet, M. Toulmonde, M. A. Burgess, V. Bolejack, D. Reinke, K. M. Wani, W.L. Wang, A. J. Lazar, C. L. Roland, J. A. Wargo, A. Italiano , C. Sautès-Fridman, H. A. Tawbi and W.H. Fridman

(addition of authors A. Bougoüin, M. Moreira, K. M. Wani and W.L. Wang)

I agree with these changes.

Best regards,

Aurélien de Reyniès

Sujet : Re: [EXT] **URGENT** Agreement needed for publication
Date : Tue, 5 Nov 2019 12:34:33 +0000
De : Keung,Emily Z. <EKeung@mdanderson.org>
Pour : Florent Petitprez <Florent.Petitprez@ligue-cancer.net>
Copie à : Echeverry,Lisa R <LEcheverry@mdanderson.org>

Hi Florent,
>
> "The list of authors of manuscript 2018-06-08855D, titled "B cells are associated with sarcoma survival and immunotherapy response", was changed from
>
> F. Petitprez, A. de Reyniès, E. Z. Keung, T.W. Chen, C.M. Sun, J. Calderaro, Y.M. Jeng, L.P. Hsiao, L. Lacroix, G. Lacroix, I. Natario, J. Adam, C. Lucchesi, Y. Laizet, M. Toulmonde, M. A. Burgess, V. Bolejack, D. Reinke, A. J. Lazar, C. L. Roland, J. A. Wargo, A. Italiano, C. Sautès-Fridman, H. A. Tawbi and W.H. Fridman
>
> to
>
> F. Petitprez, A. de Reyniès, E. Z. Keung, T.W. Chen, C.M. Sun, J. Calderaro, Y.M. Jeng, L.P. Hsiao, L. Lacroix, A. Bougoüin, M. Moreira, G. Lacroix, I. Natario, J. Adam, C. Lucchesi, Y. Laizet, M. Toulmonde, M. A. Burgess, V. Bolejack, D. Reinke, K. M. Wani, W.L. Wang, A. J. Lazar, C. L. Roland, J. A. Wargo, A. Italiano , C. Sautès-Fridman, H. A. Tawbi and W.H. Fridman
>
> (addition of authors A. Bougoüin, M. Moreira, K. M. Wani and W.L. Wang)
>
> I agree with these changes."
>
> Thank you!
-Emily

Sujet : Re: **URGENT** Agreement needed for publication
Date : Tue, 5 Nov 2019 17:14:00 +0800
De : Tom Chen <saxotomy@gmail.com>
Pour : Florent Petitprez <Florent.Petitprez@ligue-cancer.net>

I agree with the author changes
Tom Wei-Wu Chen

Sujet : Re: **URGENT** Agreement needed for publication
Date : Tue, 5 Nov 2019 14:58:36 +0100
De : Cheng-Ming Sun <cheng-ming.sun@crc.jussieu.fr>
Pour : Florent Petitprez <Florent.Petitprez@ligue-cancer.net>
Copie à : Aurélien De Reynies <Aurelien.DeReynies@ligue-cancer.net>, Keung,Emily <ekeung@mdanderson.org>, Wei-Wu (Tom) Chen <saxotomy@gmail.com>, Julien Calderaro <julien.calderaro@hmn.aphp.fr>, liippiil@yahoo.com.tw <liippiil@yahoo.com.tw>, mRNA0912@yahoo.com.tw <mRNA0912@yahoo.com.tw>, laetitia.lacroix@inserm.fr, Antoine Bougouin <antoine.bougouin@crc.jussieu.fr>, Guillaume Lacroix (guillaume.lacroix@crc.jussieu.fr) <guillaume.lacroix@crc.jussieu.fr>, Ivo Gameiro Natario <ivo.natario@gmail.com>, ADAM Julien <julien.adam@gustaveroussy.fr>, Lucchesi Carlo <c.lucchesi@bordeaux.unicancer.fr>, Laizet Yechan <y.laizet@bordeaux.unicancer.fr>, Toulmonde Maud <m.toulmonde@bordeaux.unicancer.fr>, Burgess, Melissa <burgessma@upmc.edu>, vanessab@crab.org <vanessab@crab.org>, Reinke, Denise <DReinke@sarctrials.org>, Kwani@mdanderson.org, Lazar,Alexander <alazar@mdanderson.org>, Roland,Christina Lynn <CLRoland@mdanderson.org>, Wargo,Jennifer <JWargo@mdanderson.org>, Antoine Italiano <A.Italiano@bordeaux.unicancer.fr>, Catherine Sautes-Fridman <catherine.fridman@crc.jussieu.fr>, Tawbi,Hussein <HTawbi@mdanderson.org>, Hervé Fridman <herve.fridman@crc.jussieu.fr>, Marco Moreira <Marco.moreira@outlook.fr>, wlwang@mdanderson.org

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(addition of authors A. Bougoüin, M. Moreira, K. M. Wani and W.L. Wang)

I agree with these changes.

Thank you very much

--
Cheng-Ming Sun, PhD

Cordeliers Research Center
UMRS 1138
Team 13, Cancer, Immune Control and Escape
15 Rue de l'Ecole de Médecine
75006 PARIS
cheng-ming.sun@crc.jussieu.fr

Sujet : Re: **URGENT** Agreement needed for publication
Date : Tue, 5 Nov 2019 10:05:09 +0100
De : Julien Calderaro <juliencalderaro@gmail.com>
Pour : Florent Petitprez <Florent.Petitprez@ligue-cancer.net>

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(addition of authors A. Bougoüin, M. Moreira, K. M. Wani and W.L. Wang)

I agree with these changes
Best
Julien

Sujet : Email of agreement for Nature

Date : Thu, 7 Nov 2019 16:26:28 +0800

De : Yung-Ming Jeng <mrna0912@gmail.com>

Pour : Florent.Petitprez@ligue-cancer.net

The list of authors of manuscript 2018-06-08855D, titled "B cells are associated with sarcoma survival and immunotherapy response", was changed from

F. Petitprez, A. de Reyniès, E. Z. Keung, T.W. Chen, C.M. Sun, J. Calderaro, Y.M. Jeng, L.P. Hsiao, L. Lacroix, G. Lacroix, I. Natario, J. Adam, C. Lucchesi, Y. Laizet, M. Toulmonde, M. A. Burgess, V. Bolejack, D. Reinke, A. J. Lazar, C. L. Roland, J. A. Wargo, A. Italiano, C. Sautès-Fridman, H. A. Tawbi and W.H. Fridman

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(addition of authors A. Bougoüin, M. Moreira, K. M. Wani and W.L. Wang)

I agree with these changes.

Best Regards

Yung-Ming Jeng

Sujet : Re: RE : **URGENT** Agreement needed for publication
Date : Tue, 5 Nov 2019 19:55:58 +0800
De : 莉萍 蕭 <liippiil@yahoo.com.tw>
Pour : Ivo Gameiro Natario <ivo.natario@gmail.com>
Copie à : Florent Petitprez <Florent.Petitprez@ligue-cancer.net>, Aurélien De Reynies <Aurelien.DeReynies@ligue-cancer.net>, Keung,Emily <ekeung@mdanderson.org>, Wei-Wu (Tom) Chen <saxotomy@gmail.com>, Cheng-Ming Sun <cheng-ming.sun@crc.jussieu.fr>, Julien Calderaro <julien.calderaro@hmn.aphp.fr>, mRNA0912@yahoo.com.tw <mRNA0912@yahoo.com.tw>, laetitia.lacroix@inserm.fr <laetitia.lacroix@inserm.fr>, antoine.bougouin@crc.jussieu.fr <antoine.bougouin@crc.jussieu.fr>, guillaume.lacroix@crc.jussieu.fr <guillaume.lacroix@crc.jussieu.fr>, ADAM Julien <julien.adam@gustaveroussy.fr>, Lucchesi Carlo <c.lucchesi@bordeaux.unicancer.fr>, Laizet Yechan <y.laizet@bordeaux.unicancer.fr>, Toulmonde Maud <m.toulmonde@bordeaux.unicancer.fr>, Burgess, Melissa <burgessma@upmc.edu>, vanessab@crab.org <vanessab@crab.org>, Reinke, Denise <DReinke@sarctrials.org>, Kwani@mdanderson.org <Kwani@mdanderson.org>, Lazar,Alexander <alazar@mdanderson.org>, Roland,Christina Lynn <CLRoland@mdanderson.org>, Wargo,Jennifer <JWargo@mdanderson.org>, Italiano Antoine <A.Italiano@bordeaux.unicancer.fr>, catherine fridman <catherine.fridman@crc.jussieu.fr>, Tawbi,Hussein <HTawbi@mdanderson.org>, FRIDMAN Hervé <herve.fridman@crc.jussieu.fr>, marco moreira <Marco.moreira@outlook.fr>, wlwang@mdanderson.org <wlwang@mdanderson.org>

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(addition of authors A. Bougoüin, M. Moreira, K. M. Wani and W.L. Wang)

I agree with these changes."

Best Regards,

Li-Ping Hsiao

Sujet : RE: **URGENT** Agreement needed for publication
Date : Tue, 5 Nov 2019 10:05:44 +0100
De : laetitia.lacroix@inserm.fr
Pour : 'Florent Petitprez' <Florent.Petitprez@ligue-cancer.net>

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(addition of authors A. Bougoüin, M. Moreira, K. M. Wani and W.L. Wang)

I agree with these changes."

Laetitia Lacroix

IMRB-U955-Equipe 9

"Immunologie et oncogénèse des tumeurs lymphoïdes"

Hôpital Henri Mondor

51 avenue du Mal De Lattre de Tassigny

94010 CRETEIL

Tél. : 01 49 81 37 68

Sujet : Agreement needed for publication
Date : Tue, 5 Nov 2019 10:15:50 +0100
De : Antoine BOUGOUIN <antoine.bougouin@upmc.fr>
Pour : Florent Petitprez <Florent.Petitprez@ligue-cancer.net>

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F. Petitprez, A. de Reyniès, E. Z. Keung, T.W. Chen, C.M. Sun, J. Calderaro, Y.M. Jeng, L.P. Hsiao, L. Lacroix, A. Bougoüin, M. Moreira, G. Lacroix, I. Natario, J. Adam, C. Lucchesi, Y. Laizet, M. Toulmonde, M. A. Burgess, V. Bolejack, D. Reinke, K. M. Wani, W.L. Wang, A. J. Lazar, C. L. Roland, J. A. Wargo, A. Italiano , C. Sautès-Fridman, H. A. Tawbi and W.H. Fridman
(addition of authors A. Bougoüin, M. Moreira, K. M. Wani and W.L. Wang)

I agree with these changes."

Antoine BOUGOUIN

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Sujet : Re: **URGENT** Agreement needed for publication
Date : Tue, 5 Nov 2019 09:39:49 +0000
De : marco moreira <marco.moreira@outlook.fr>
Pour : Florent Petitprez <Florent.Petitprez@ligue-cancer.net>

Dear Florent,

The list of authors of manuscript 2018-06-08855D, titled "B cells are associated with sarcoma survival and immunotherapy response", was changed from
F. Petitprez, A. de Reyniès, E. Z. Keung, T.W. Chen, C.M. Sun, J. Calderaro, Y.M. Jeng, L.P. Hsiao, L. Lacroix, G. Lacroix, I. Natario, J. Adam, C. Lucchesi, Y. Laizet, M. Toulmonde, M. A. Burgess, V. Bolejack, D. Reinke, A. J. Lazar, C. L. Roland, J. A. Wargo, A. Italiano, C. Sautès-Fridman, H. A. Tawbi and W.H. Fridman
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(addition of authors A. Bougoüin, M. Moreira, K. M. Wani and W.L. Wang)

I agree with these changes.

Best regards,

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Sujet : Re: **URGENT** Agreement needed for publication
Date : Tue, 5 Nov 2019 10:09:48 +0100
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Pour : Florent Petitprez <Florent.Petitprez@ligue-cancer.net>

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(addition of authors A. Bougoüin, M. Moreira, K. M. Wani and W.L. Wang)

I agree with these changes.

Guillaume Lacroix
Assistant Engineer

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Sujet : RE : **URGENT** Agreement needed for publication
Date : Tue, 5 Nov 2019 11:51:06 +0100
De : Ivo Gameiro Natario <ivo.natario@gmail.com>
Pour : Florent Petitprez <Florent.Petitprez@ligue-cancer.net>, Aurélien De Reynies <Aurelien.DeReynies@ligue-cancer.net>, Keung,Emily <ekeung@mdanderson.org>, Wei-Wu (Tom) Chen <saxotomy@gmail.com>, Cheng-Ming Sun <cheng-ming.sun@crc.jussieu.fr>, Julien Calderaro <julien.calderaro@hmn.aphp.fr>, liippiil@yahoo.com.tw <liippiil@yahoo.com.tw>, mRNA0912@yahoo.com.tw <mRNA0912@yahoo.com.tw>, laetitia.lacroix@inserm.fr <laetitia.lacroix@inserm.fr>, antoine.bougouin@crc.jussieu.fr <antoine.bougouin@crc.jussieu.fr>, guillaume.lacroix@crc.jussieu.fr <guillaume.lacroix@crc.jussieu.fr>, ADAM Julien <julien.adam@gustaveroussy.fr>, Lucchesi Carlo <c.lucchesi@bordeaux.unicancer.fr>, Laizet Yechan <y.laizet@bordeaux.unicancer.fr>, Toulmonde Maud <m.toulmonde@bordeaux.unicancer.fr>, Burgess, Melissa <burgessma@upmc.edu>, vanessab@crab.org <vanessab@crab.org>, Reinke, Denise <DReinke@sarctrials.org>, Kwani@mdanderson.org <Kwani@mdanderson.org>, Lazar,Alexander <alazar@mdanderson.org>, Roland,Christina Lynn <CLRoland@mdanderson.org>, Wargo,Jennifer <JWargo@mdanderson.org>, Italiano Antoine <A.Italiano@bordeaux.unicancer.fr>, catherine fridman <catherine.fridman@crc.jussieu.fr>, Tawbi,Hussein <HTawbi@mdanderson.org>, FRIDMAN Hervé <herve.fridman@crc.jussieu.fr>, marco moreira <Marco.moreira@outlook.fr>, wlwang@mdanderson.org <wlwang@mdanderson.org>

Dear Florent,

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F. Petitprez, A. de Reyniès, E. Z. Keung, T.W. Chen, C.M. Sun, J. Calderaro, Y.M. Jeng, L.P. Hsiao, L. Lacroix, G. Lacroix, I. Natario, J. Adam, C. Lucchesi, Y. Laizet, M. Toulmonde, M. A. Burgess, V. Bolejack, D. Reinke, A. J. Lazar, C. L. Roland, J. A. Wargo, A. Italiano, C. Sautès-Fridman, H. A. Tawbi and W.H. Fridman

to

F. Petitprez, A. de Reyniès, E. Z. Keung, T.W. Chen, C.M. Sun, J. Calderaro, Y.M. Jeng, L.P. Hsiao, L. Lacroix, A. Bougoüin, M. Moreira, G. Lacroix, I. Natario, J. Adam, C. Lucchesi, Y. Laizet, M. Toulmonde, M. A. Burgess, V. Bolejack, D. Reinke, K. M. Wani, W.L. Wang, A. J. Lazar, C. L. Roland, J. A. Wargo, A. Italiano , C. Sautès-Fridman, H. A. Tawbi and W.H. Fridman

(addition of authors A. Bougoüin, M. Moreira, K. M. Wani and W.L. Wang)

I agree with these changes.

Best regards,

Ivo Natario

Sujet : RE:**URGENT** Agreement needed for publication
Date : Tue, 5 Nov 2019 20:33:31 +0000
De : ADAM Julien <Julien.ADAM@gustaveroussy.fr>
Pour : Florent Petitprez <Florent.Petitprez@ligue-cancer.net>

Dear Florent,

The list of authors of manuscript 2018-06-08855D, titled "B cells are associated with sarcoma survival and immunotherapy response", was changed from

F. Petitprez, A. de Reyniès, E. Z. Keung, T.W. Chen, C.M. Sun, J. Calderaro, Y.M. Jeng, L.P. Hsiao, L. Lacroix, G. Lacroix, I. Natario, J. Adam, C. Lucchesi, Y. Laizet, M. Toulmonde, M. A. Burgess, V. Bolejack, D. Reinke, A. J. Lazar, C. L. Roland, J. A. Wargo, A. Italiano, C. Sautès-Fridman, H. A. Tawbi and W.H. Fridman

to

F. Petitprez, A. de Reyniès, E. Z. Keung, T.W. Chen, C.M. Sun, J. Calderaro, Y.M. Jeng, L.P. Hsiao, L. Lacroix, A. Bougoüin, M. Moreira, G. Lacroix, I. Natario, J. Adam, C. Lucchesi, Y. Laizet, M. Toulmonde, M. A. Burgess, V. Bolejack, D. Reinke, K. M. Wani, W.L. Wang, A. J. Lazar, C. L. Roland, J. A. Wargo, A. Italiano , C. Sautès-Fridman, H. A. Tawbi and W.H. Fridman

(addition of authors A. Bougoüin, M. Moreira, K. M. Wani and W.L. Wang)

I agree with these changes.

Julien Adam

Sujet : Re: **URGENT** Agreement needed for publication
Date : Tue, 5 Nov 2019 15:04:28 +0000
De : Lucchesi Carlo <c.lucchesi@bordeaux.unicancer.fr>
Pour : Florent Petitprez <Florent.Petitprez@ligue-cancer.net>

The list of authors of manuscript 2018-06-08855D, titled "B cells are associated with sarcoma survival and immunotherapy response", was changed from

F. Petitprez, A. de Reyniès, E. Z. Keung, T.W. Chen, C.M. Sun, J. Calderaro, Y.M. Jeng, L.P. Hsiao, L. Lacroix, G. Lacroix, I. Natario, J. Adam, C. Lucchesi, Y. Laizet, M. Toulmonde, M. A. Burgess, V. Bolejack, D. Reinke, A. J. Lazar, C. L. Roland, J. A. Wargo, A. Italiano, C. Sautès-Fridman, H. A. Tawbi and W.H. Fridman

to

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(addition of authors A. Bougoüin, M. Moreira, K. M. Wani and W.L. Wang)

I agree with these changes.

Sujet : RE:**URGENT** Agreement needed for publication
Date : Tue, 5 Nov 2019 09:47:08 +0000
De : Laizet Yechan <y.laizet@bordeaux.unicancer.fr>
Pour : Florent Petitprez <Florent.Petitprez@ligue-cancer.net>, Aurélien De Reynies <Aurelien.DeReynies@ligue-cancer.net>, Keung,Emily <ekeung@mdanderson.org>, Wei-Wu (Tom) Chen <saxotomy@gmail.com>, Cheng-Ming Sun <cheng-ming.sun@crc.jussieu.fr>, Julien Calderaro <julien.calderaro@hmn.aphp.fr>, liippiil@yahoo.com.tw <liippiil@yahoo.com.tw>, mRNA0912@yahoo.com.tw <mRNA0912@yahoo.com.tw>, laetitia.lacroix@inserm.fr <laetitia.lacroix@inserm.fr>, antoine.bougouin@crc.jussieu.fr <antoine.bougouin@crc.jussieu.fr>, guillaume.lacroix@crc.jussieu.fr <guillaume.lacroix@crc.jussieu.fr>, Ivo Gameiro Natario <ivo.natario@gmail.com>, ADAM Julien <julien.adam@gustaveroussy.fr>, Lucchesi Carlo <c.lucchesi@bordeaux.unicancer.fr>, Toulmonde Maud <m.toulmonde@bordeaux.unicancer.fr>, Burgess, Melissa <burgessma@upmc.edu>, vanessab@crab.org <vanessab@crab.org>, Reinke, Denise <DReinke@sarctrials.org>, Kwani@mdanderson.org <Kwani@mdanderson.org>, Lazar,Alexander <alazar@mdanderson.org>, Roland,Christina Lynn <CLRoland@mdanderson.org>, Wargo,Jennifer <JWargo@mdanderson.org>, Italiano Antoine <A.Italiano@bordeaux.unicancer.fr>, catherine fridman <catherine.fridman@crc.jussieu.fr>, Tawbi,Hussein <HTawbi@mdanderson.org>, FRIDMAN Hervé <herve.fridman@crc.jussieu.fr>, marco moreira <Marco.moreira@outlook.fr>, wlwang@mdanderson.org <wlwang@mdanderson.org>

Hello FLorent,

The list of authors of manuscript 2018-06-08855D, titled "B cells are associated with sarcoma survival and immunotherapy response", was changed from
F. Petitprez, A. de Reyniès, E. Z. Keung, T.W. Chen, C.M. Sun, J. Calderaro, Y.M. Jeng, L.P. Hsiao, L. Lacroix, G. Lacroix, I. Natario, J. Adam, C. Lucchesi, Y. Laizet, M. Toulmonde, M. A. Burgess, V. Bolejack, D. Reinke, A. J. Lazar, C. L. Roland, J. A. Wargo, A. Italiano, C. Sautès-Fridman, H. A. Tawbi and W.H. Fridman
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(addition of authors A. Bougoüin, M. Moreira, K. M. Wani and W.L. Wang)
I agree with these changes.
Regards.

Yec'han

Yec'han LAIZET
Institut Bergonié, Bioinformatics
y.laizet@bordeaux.unicancer.fr
Poste : 44 24
Tel : +33 (0)5 56 33 04 24
229, cours de l'Argonne, 33000 BORDEAUX

Sujet : RE: **URGENT** Agreement needed for publication
Date : Tue, 5 Nov 2019 14:43:57 +0000
De : Toulmonde Maud <m.toulmonde@bordeaux.unicancer.fr>
Pour : 'Florent Petitprez' <Florent.Petitprez@ligue-cancer.net>

Dear Florent,

The list of authors of manuscript 2018-06-08855D, titled "B cells are associated with sarcoma survival and immunotherapy response", was changed from

F. Petitprez, A. de Reyniès, E. Z. Keung, T.W. Chen, C.M. Sun, J. Calderaro, Y.M. Jeng, L.P. Hsiao, L. Lacroix, G. Lacroix, I. Natario, J. Adam, C. Lucchesi, Y. Laizet, M. Toulmonde, M. A. Burgess, V. Bolejack, D. Reinke, A. J. Lazar, C. L. Roland, J. A. Wargo, A. Italiano, C. Sautès-Fridman, H. A. Tawbi and W.H. Fridman

to

F. Petitprez, A. de Reyniès, E. Z. Keung, T.W. Chen, C.M. Sun, J. Calderaro, Y.M. Jeng, L.P. Hsiao, L. Lacroix, A. Bougoüin, M. Moreira, G. Lacroix, I. Natario, J. Adam, C. Lucchesi, Y. Laizet, M. Toulmonde, M. A. Burgess, V. Bolejack, D. Reinke, K. M. Wani, W.L. Wang, A. J. Lazar, C. L. Roland, J. A. Wargo, A. Italiano , C. Sautès-Fridman, H. A. Tawbi and W.H. Fridman

(addition of authors A. Bougoüin, M. Moreira, K. M. Wani and W.L. Wang)

I agree with these changes.

Maud TOULMONDE, MD

Institut Bergonié, Medical Oncology Dept

Early Phase Trials and Sarcoma Units

229 cours de l'Argonne

33076 Bordeaux Cedex France

Tel : + 33 5 47 30 60 88
+ 33 6 18 47 19 79
Fax : + 33 5 47 30 60 83

m.toulmonde@bordeaux.unicancer.fr

Sujet : RE: **URGENT** Agreement needed for publication
Date : Tue, 5 Nov 2019 11:53:23 +0000
De : Burgess, Melissa <burgessma@upmc.edu>
Pour : 'Florent Petitprez' <Florent.Petitprez@ligue-cancer.net>

Dear Florent,

The list of authors of manuscript 2018-06-08855D, titled "B cells are associated with sarcoma survival and immunotherapy response", was changed from

F. Petitprez, A. de Reyniès, E. Z. Keung, T.W. Chen, C.M. Sun, J. Calderaro, Y.M. Jeng, L.P. Hsiao, L. Lacroix, G. Lacroix, I. Natario, J. Adam, C. Lucchesi, Y. Laizet, M. Toulmonde, M. A. Burgess, V. Bolejack, D. Reinke, A. J. Lazar, C. L. Roland, J. A. Wargo, A. Italiano, C. Sautès-Fridman, H. A. Tawbi and W.H. Fridman

to

F. Petitprez, A. de Reyniès, E. Z. Keung, T.W. Chen, C.M. Sun, J. Calderaro, Y.M. Jeng, L.P. Hsiao, L. Lacroix, A. Bougoüin, M. Moreira, G. Lacroix, I. Natario, J. Adam, C. Lucchesi, Y. Laizet, M. Toulmonde, M. A. Burgess, V. Bolejack, D. Reinke, K. M. Wani, W.L. Wang, A. J. Lazar, C. L. Roland, J. A. Wargo, A. Italiano , C. Sautès-Fridman, H. A. Tawbi and W.H. Fridman

(addition of authors A. Bougoüin, M. Moreira, K. M. Wani and W.L. Wang)

I agree with these changes.

Sincerely,

Melissa

Melissa Burgess

Assistant Professor of Medicine
University of Pittsburgh, Department of Medicine, Division of Hematology/Oncology

5150 Centre Avenue, Fifth Floor
Pittsburgh, PA 15232
T 412-623-7277

F 412-648-6579

Email burgessma@upmc.edu

Sujet : RE: **URGENT** Agreement needed for publication
Date : Tue, 5 Nov 2019 15:44:01 +0000
De : vanessab (Vanessa Bolejack) <vanessab@crab.org>
Pour : Florent Petitprez <Florent.Petitprez@ligue-cancer.net>

Hello,

Regarding the following,

"The list of authors of manuscript 2018-06-08855D, titled "B cells are associated with sarcoma survival and immunotherapy response", was changed from

F. Petitprez, A. de Reyniès, E. Z. Keung, T.W. Chen, C.M. Sun, J. Calderaro, Y.M. Jeng, L.P. Hsiao, L. Lacroix, G. Lacroix, I. Natario, J. Adam, C. Lucchesi, Y. Laizet, M. Toulmonde, M. A. Burgess, V. Bolejack, D. Reinke, A. J. Lazar, C. L. Roland, J. A. Wargo, A. Italiano, C. Sautès-Fridman, H. A. Tawbi and W.H. Fridman

to

F. Petitprez, A. de Reyniès, E. Z. Keung, T.W. Chen, C.M. Sun, J. Calderaro, Y.M. Jeng, L.P. Hsiao, L. Lacroix, A. Bougoüin, M. Moreira, G. Lacroix, I. Natario, J. Adam, C. Lucchesi, Y. Laizet, M. Toulmonde, M. A. Burgess, V. Bolejack, D. Reinke, K. M. Wani, W.L. Wang, A. J. Lazar, C. L. Roland, J. A. Wargo, A. Italiano , C. Sautès-Fridman, H. A. Tawbi and W.H. Fridman

(addition of authors A. Bougoüin, M. Moreira, K. M. Wani and W.L. Wang)

I agree with these changes.

Best Regards,

Vanessa Bolejack

Vanessa Bolejack, MPH

Biostatistician Consultant

Cancer Research and Biostatistics

Sujet : RE: **URGENT** Agreement needed for publication
Date : Tue, 5 Nov 2019 12:23:02 +0000
De : Reinke, Denise <DReinke@sarctrials.org>
Pour : Florent Petitprez <Florent.Petitprez@ligue-cancer.net>, Aurélien De Reynies <Aurelien.DeReynies@ligue-cancer.net>, Keung,Emily <ekeung@mdanderson.org>, Wei-Wu (Tom) Chen <saxotomy@gmail.com>, Cheng-Ming Sun <cheng-ming.sun@crc.jussieu.fr>, Julien Calderaro <julien.calderaro@hmn.aphp.fr>, liippiil@yahoo.com.tw <liippiil@yahoo.com.tw>, mRNA0912@yahoo.com.tw <mRNA0912@yahoo.com.tw>, laetitia.lacroix@inserm.fr <laetitia.lacroix@inserm.fr>, antoine.bougouin@crc.jussieu.fr <antoine.bougouin@crc.jussieu.fr>, guillaume.lacroix@crc.jussieu.fr <guillaume.lacroix@crc.jussieu.fr>, Ivo Gameiro Natario <ivo.natario@gmail.com>, ADAM Julien <julien.adam@gustaveroussy.fr>, Lucchesi Carlo <c.lucchesi@bordeaux.unicancer.fr>, Laizet Yechan <y.laizet@bordeaux.unicancer.fr>, Toulmonde Maud <m.toulmonde@bordeaux.unicancer.fr>, Burgess, Melissa <burgessma@upmc.edu>, vanessab@crab.org <vanessab@crab.org>, Kwani@mdanderson.org <Kwani@mdanderson.org>, Lazar,Alexander <alazar@mdanderson.org>, Roland,Christina Lynn <CLRoland@mdanderson.org>, Wargo,Jennifer <JWargo@mdanderson.org>, Italiano Antoine <A.Italiano@bordeaux.unicancer.fr>, catherine fridman <catherine.fridman@crc.jussieu.fr>, Tawbi,Hussein <HTawbi@mdanderson.org>, FRIDMAN Hervé <herve.fridman@crc.jussieu.fr>, marco moreira <Marco.moreira@outlook.fr>, wlwang@mdanderson.org <wlwang@mdanderson.org>

I am in agreement with the change

Kindly,

Denise Reinke

Sujet : RE: [EXT] Re: **URGENT** Agreement needed for publication

Date : Wed, 6 Nov 2019 16:56:25 +0000

De : Wani,Khalida M <KWani@mdanderson.org>

Pour : Florent Petitprez <Florent.Petitprez@ligue-cancer.net>, Wang,Wei-Lien <wlwang@mdanderson.org>

Dear Florent,

The list of authors of manuscript 2018-06-08855D, titled "B cells are associated with sarcoma survival and immunotherapy response", was changed from

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to

F. Petitprez, A. de Reyniès, E. Z. Keung, T.W. Chen, C.M. Sun, J. Calderaro, Y.M. Jeng, L.P. Hsiao, L. Lacroix, A. Bougoüin, M. Moreira, G. Lacroix, I. Natario, J. Adam, C. Lucchesi, Y. Laizet, M. Toulmonde, M. A. Burgess, V. Bolejack, D. Reinke, K. M. Wani, W.L. Wang, A. J. Lazar, C. L. Roland, J. A. Wargo, A. Italiano , C. Sautès-Fridman, H. A. Tawbi and W.H. Fridman

(addition of authors A. Bougoüin, M. Moreira, K. M. Wani and W.L. Wang)

I agree with these changes.

Best regards,

Khalida Wani

Sujet : RE: [EXT] **URGENT** Agreement needed for publication

Date : Thu, 7 Nov 2019 13:23:22 +0000

De : Wang,Wei-Lien <wlwang@mdanderson.org>

Pour : Florent Petitprez <Florent.Petitprez@ligue-cancer.net>

The list of authors of manuscript 2018-06-08855D, titled "B cells are associated with sarcoma survival and immunotherapy response", was changed from

F. Petitprez, A. de Reyniès, E. Z. Keung, T.W. Chen, C.M. Sun, J. Calderaro, Y.M. Jeng, L.P. Hsiao, L. Lacroix, G. Lacroix, I. Natario, J. Adam, C. Lucchesi, Y. Laizet, M. Toulmonde, M. A. Burgess, V. Bolejack, D. Reinke, A. J. Lazar, C. L. Roland, J. A. Wargo, A. Italiano, C. Sautès-Fridman, H. A. Tawbi and W.H. Fridman

to

F. Petitprez, A. de Reyniès, E. Z. Keung, T.W. Chen, C.M. Sun, J. Calderaro, Y.M. Jeng, L.P. Hsiao, L. Lacroix, A. Bougoüin, M. Moreira, G. Lacroix, I. Natario, J. Adam, C. Lucchesi, Y. Laizet, M. Toulmonde, M. A. Burgess, V. Bolejack, D. Reinke, K. M. Wani, W.L. Wang, A. J. Lazar, C. L. Roland, J. A. Wargo, A. Italiano , C. Sautès-Fridman, H. A. Tawbi and W.H. Fridman

(addition of authors A. Bougoüin, M. Moreira, K. M. Wani and W.L. Wang)

I agree with these changes.

Sujet : Alteration of Author list
Date : Tue, 5 Nov 2019 14:43:10 +0000
De : Lazar,Alexander <alazar@mdanderson.org>
Pour : 'Florent Petitprez' <Florent.Petitprez@ligue-cancer.net>

Hi Florent:

The list of authors of our manuscript 2018-06-08855D, titled "B cells are associated with sarcoma survival and immunotherapy response" was changed from:

F. Petitprez, A. de Reyniès, E. Z. Keung, T.W. Chen, C.M. Sun, J. Calderaro, Y.M. Jeng, L.P. Hsiao, L. Lacroix, G. Lacroix, I. Natario, J. Adam, C. Lucchesi, Y. Laizet, M. Toulmonde, M. A. Burgess, V. Bolejack, D. Reinke, A. J. Lazar, C. L. Roland, J. A. Wargo, A. Italiano, C. Sautès-Fridman, H. A. Tawbi and W.H. Fridman

to:

F. Petitprez, A. de Reyniès, E. Z. Keung, T.W. Chen, C.M. Sun, J. Calderaro, Y.M. Jeng, L.P. Hsiao, L. Lacroix, A. Bougoüin, M. Moreira, G. Lacroix, I. Natario, J. Adam, C. Lucchesi, Y. Laizet, M. Toulmonde, M. A. Burgess, V. Bolejack, D. Reinke, K. M. Wani, W.L. Wang, A. J. Lazar, C. L. Roland, J. A. Wargo, A. Italiano , C. Sautès-Fridman, H. A. Tawbi and W.H. Fridman

(addition of authors A. Bougoüin, M. Moreira, K. M. Wani and W.L. Wang)

I agree with these changes as these four authors made important contributions and also have reviewed and approved of the final accepted version of the manuscript.

Best,

Alex

Sujet : Re: [EXT] **URGENT** Agreement needed for publication
Date : Tue, 5 Nov 2019 11:51:56 +0000
De : Roland,Christina Lynn <CLRoland@mdanderson.org>
Pour : Florent Petitprez <Florent.Petitprez@ligue-cancer.net>

Dear Florent,

"The list of authors of manuscript 2018-06-08855D, titled "B cells are associated with sarcoma survival and immunotherapy response", was changed from

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to

F. Petitprez, A. de Reyniès, E. Z. Keung, T.W. Chen, C.M. Sun, J. Calderaro, Y.M. Jeng, L.P. Hsiao, L. Lacroix, A. Bougoüin, M. Moreira, G. Lacroix, I. Natario, J. Adam, C. Lucchesi, Y. Laizet, M. Toulmonde, M. A. Burgess, V. Bolejack, D. Reinke, K. M. Wani, W.L. Wang, A. J. Lazar, C. L. Roland, J. A. Wargo, A. Italiano , C. Sautès-Fridman, H. A. Tawbi and W.H. Fridman

(addition of authors A. Bougoüin, M. Moreira, K. M. Wani and W.L. Wang)

I agree with these changes."

Best Regards,

Christina Roland

Sujet : FW: [EXT] Re: **URGENT** Agreement needed for publication
Date : Tue, 5 Nov 2019 14:45:47 +0000
De : Wargo,Jennifer <JWargo@mdanderson.org>
Pour : 'Florent Petitprez' <Florent.Petitprez@ligue-cancer.net>

Dear Florent

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F. Petitprez, A. de Reyniès, E. Z. Keung, T.W. Chen, C.M. Sun, J. Calderaro, Y.M. Jeng, L.P. Hsiao, L. Lacroix, G. Lacroix, I. Natario, J. Adam, C. Lucchesi, Y. Laizet, M. Toulmonde, M. A. Burgess, V. Bolejack, D. Reinke, A. J. Lazar, C. L. Roland, J. A. Wargo, A. Italiano, C. Sautès-Fridman, H. A. Tawbi and W.H. Fridman
to
F. Petitprez, A. de Reyniès, E. Z. Keung, T.W. Chen, C.M. Sun, J. Calderaro, Y.M. Jeng, L.P. Hsiao, L. Lacroix, A. Bougoüin, M. Moreira, G. Lacroix, I. Natario, J. Adam, C. Lucchesi, Y. Laizet, M. Toulmonde, M. A. Burgess, V. Bolejack, D. Reinke, K. M. Wani, W.L. Wang, A. J. Lazar, C. L. Roland, J. A. Wargo, A. Italiano , C. Sautès-Fridman, H. A. Tawbi and W.H. Fridman
(addition of authors A. Bougoüin, M. Moreira, K. M. Wani and W.L. Wang)

I agree with these changes.

Thank you very much

Jennifer Wargo

Sujet : RE:**URGENT** Agreement needed for publication
Date : Tue, 5 Nov 2019 09:44:42 +0000
De : Italiano Antoine <A.Italiano@bordeaux.unicancer.fr>
Pour : Florent Petitprez <Florent.Petitprez@ligue-cancer.net>

The list of authors of manuscript 2018-06-08855D, titled "B cells are associated with sarcoma survival and immunotherapy response", was changed from

F. Petitprez, A. de Reyniès, E. Z. Keung, T.W. Chen, C.M. Sun, J. Calderaro, Y.M. Jeng, L.P. Hsiao, L. Lacroix, G. Lacroix, I. Natario, J. Adam, C. Lucchesi, Y. Laizet, M. Toulmonde, M. A. Burgess, V. Bolejack, D. Reinke, A. J. Lazar, C. L. Roland, J. A. Wargo, A. Italiano, C. Sautès-Fridman, H. A. Tawbi and W.H. Fridman

to

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(addition of authors A. Bougoüin, M. Moreira, K. M. Wani and W.L. Wang)

I agree with these changes."

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Sujet : Re: **URGENT** Agreement needed for publication
Date : Tue, 5 Nov 2019 12:44:51 +0100
De : Catherine Sautes Fridman <catherine.fridman@crc.jussieu.fr>
Pour : Florent Petitprez <Florent.Petitprez@ligue-cancer.net>

Dear Florent

"The list of authors of manuscript 2018-06-08855D, titled "B cells are associated with sarcoma survival and immunotherapy response", was changed from

F. Petitprez, A. de Reyniès, E. Z. Keung, T.W. Chen, C.M. Sun, J. Calderaro, Y.M. Jeng, L.P. Hsiao, L. Lacroix, G. Lacroix, I. Natario, J. Adam, C. Lucchesi, Y. Laizet, M. Toulmonde, M. A. Burgess, V. Bolejack, D. Reinke, A. J. Lazar, C. L. Roland, J. A. Wargo, A. Italiano, C. Sautès-Fridman, H. A. Tawbi and W.H. Fridman

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I agree with these changes."
Best Regards,

catherine sautès-Fridman

Sujet : Re: [EXT] **URGENT** Agreement needed for publication
Date : Tue, 5 Nov 2019 14:04:28 +0000
De : Tawbi,Hussein <HTawbi@mdanderson.org>
Pour : Florent Petitprez <Florent.Petitprez@ligue-cancer.net>

Dear Florent,

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Best,
Hussein
Hussein Tawbi, MD, PhD | Associate Professor
Deputy Chair, Department of Melanoma Medical Oncology
Director of Melanoma Clinical Research & Early Drug Development
Co-Director, MD Anderson Brain Metastasis Clinic
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UT MD Anderson Cancer Center | 1400 Holcombe Blvd. | Houston, TX 77030
HTawbi@mdanderson.org (713-745-7763 phone | 6 713-745-1046 fax

TCGA SARC
n=213

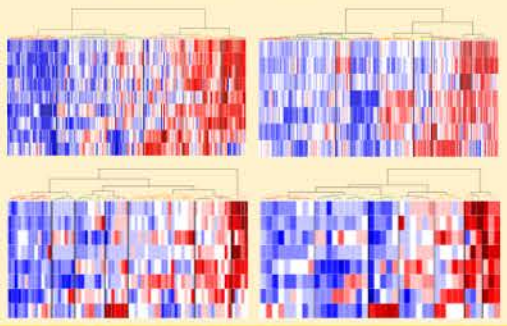
GSE21050
n=283

GSE21122
n=72

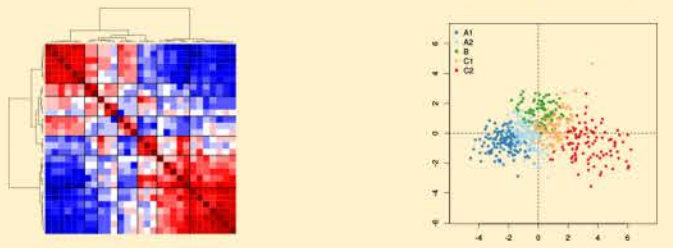
GSE30929
n=40

Discovery cohort (n=608)

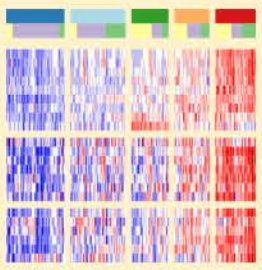
TME EVALUATION AND CLASSIFICATION



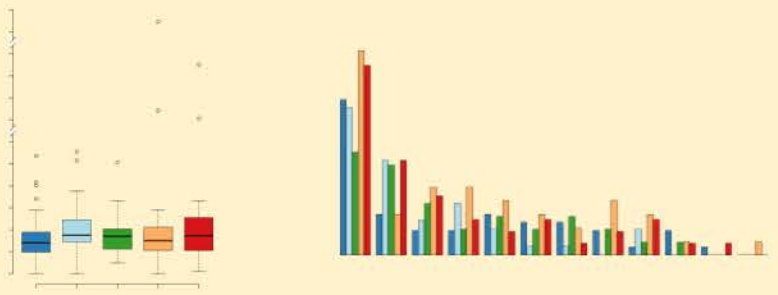
ASSESSMENT AND PREDICTION OF CONSENSUS CLUSTERS: SARCOMA IMMUNE CLASSES (SIC)



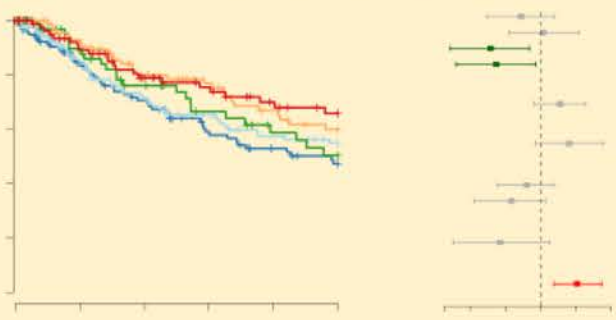
CHARACTERIZATION OF THE CLASSES



MOLECULAR CHARACTERISTICS OF SIC



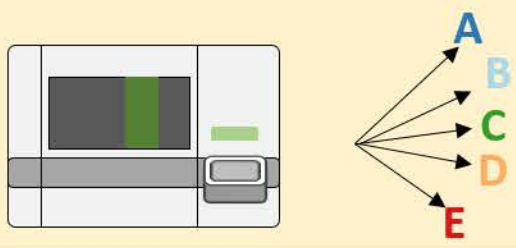
SURVIVAL



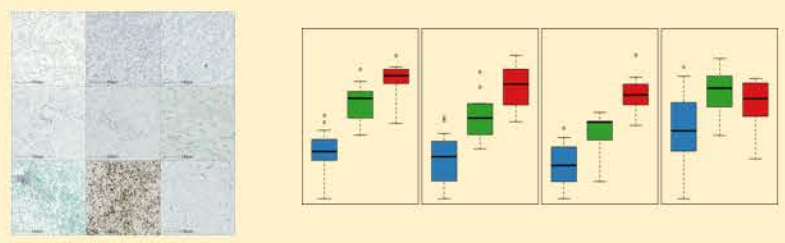
FSG
n=168

NTUH
n=93

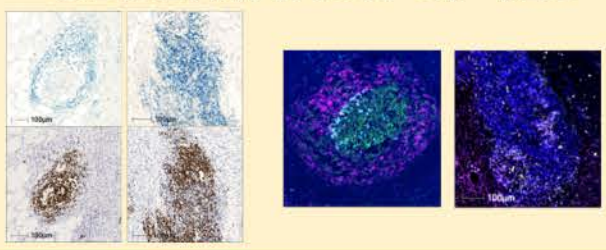
IMMUNE CLASS PREDICTION



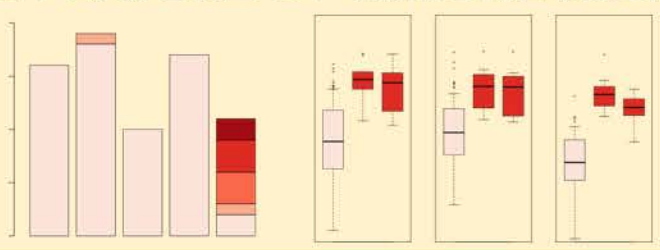
VALIDATION OF THE SIC PROFILES



OBSERVATION OF TLS



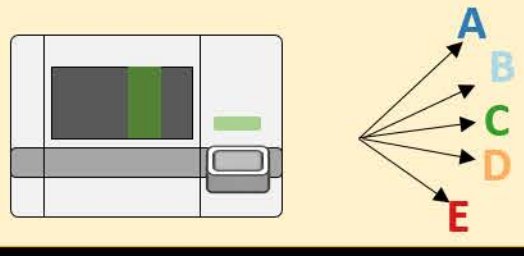
TLS FEATURE OF IMMUNE HIGH SIC



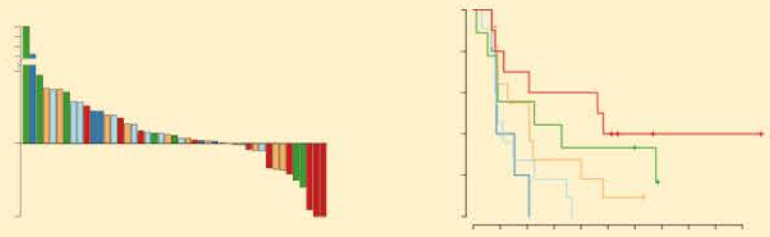
Anti PD-1

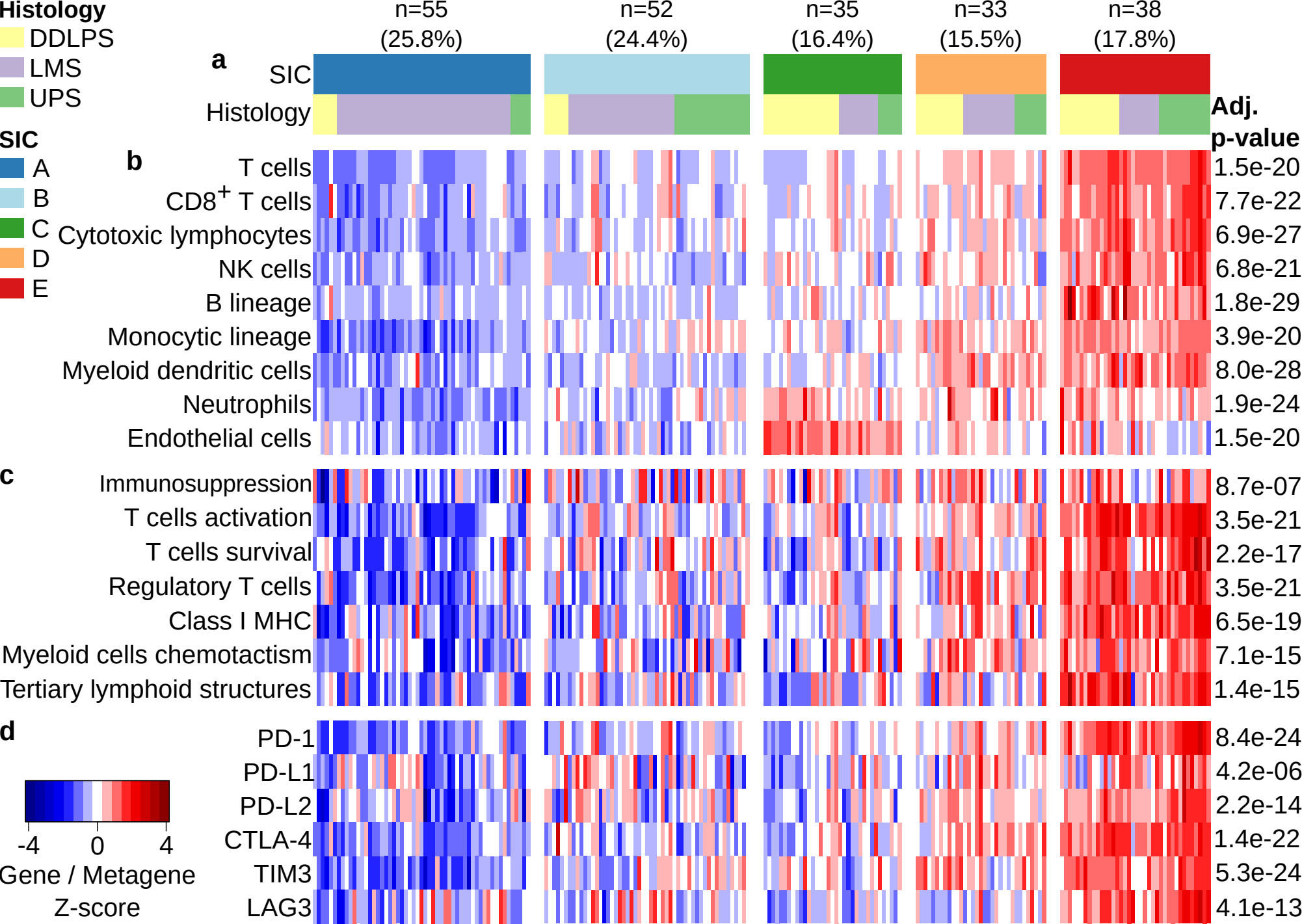
SARC028
n=47

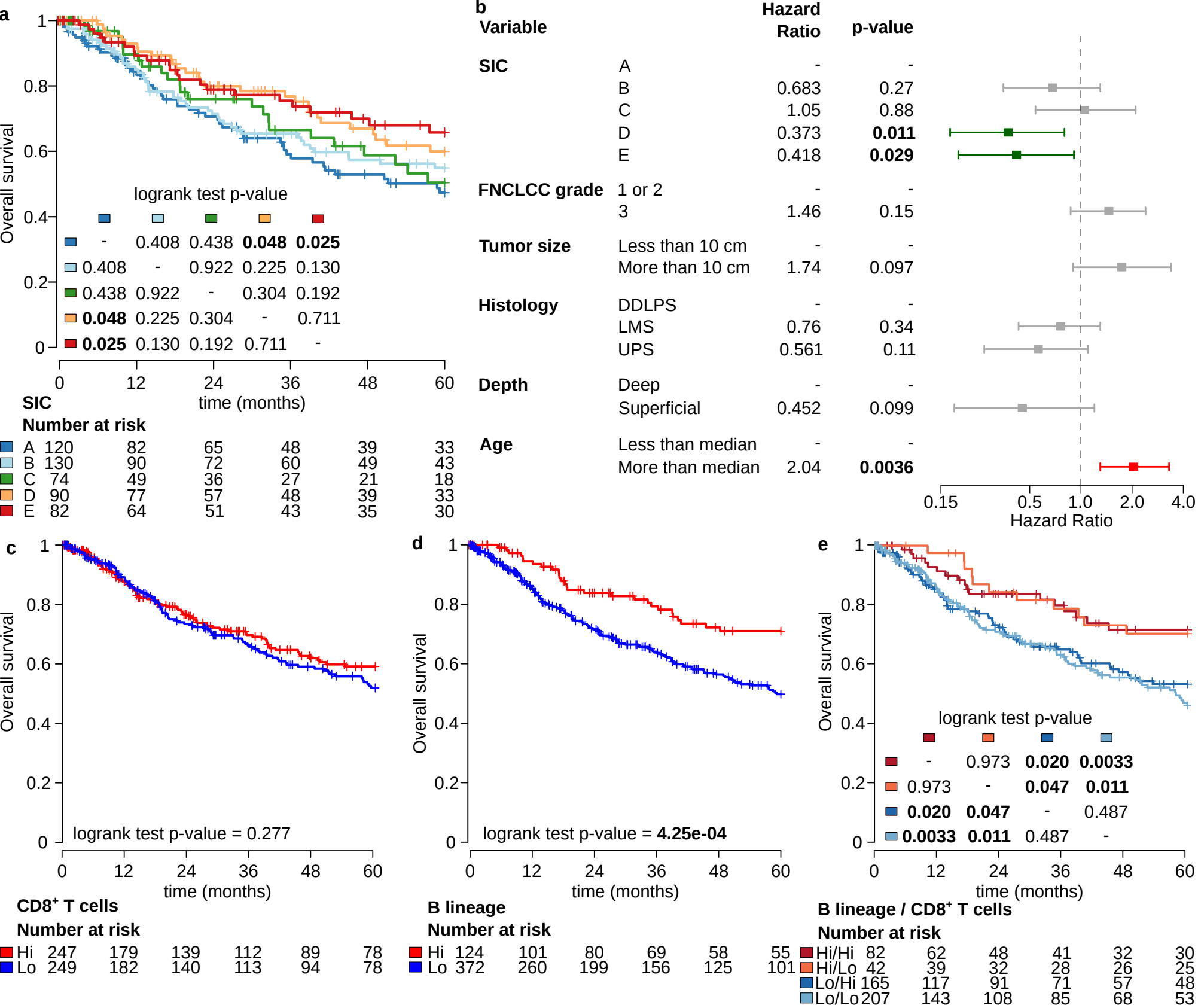
IMMUNE CLASS PREDICTION

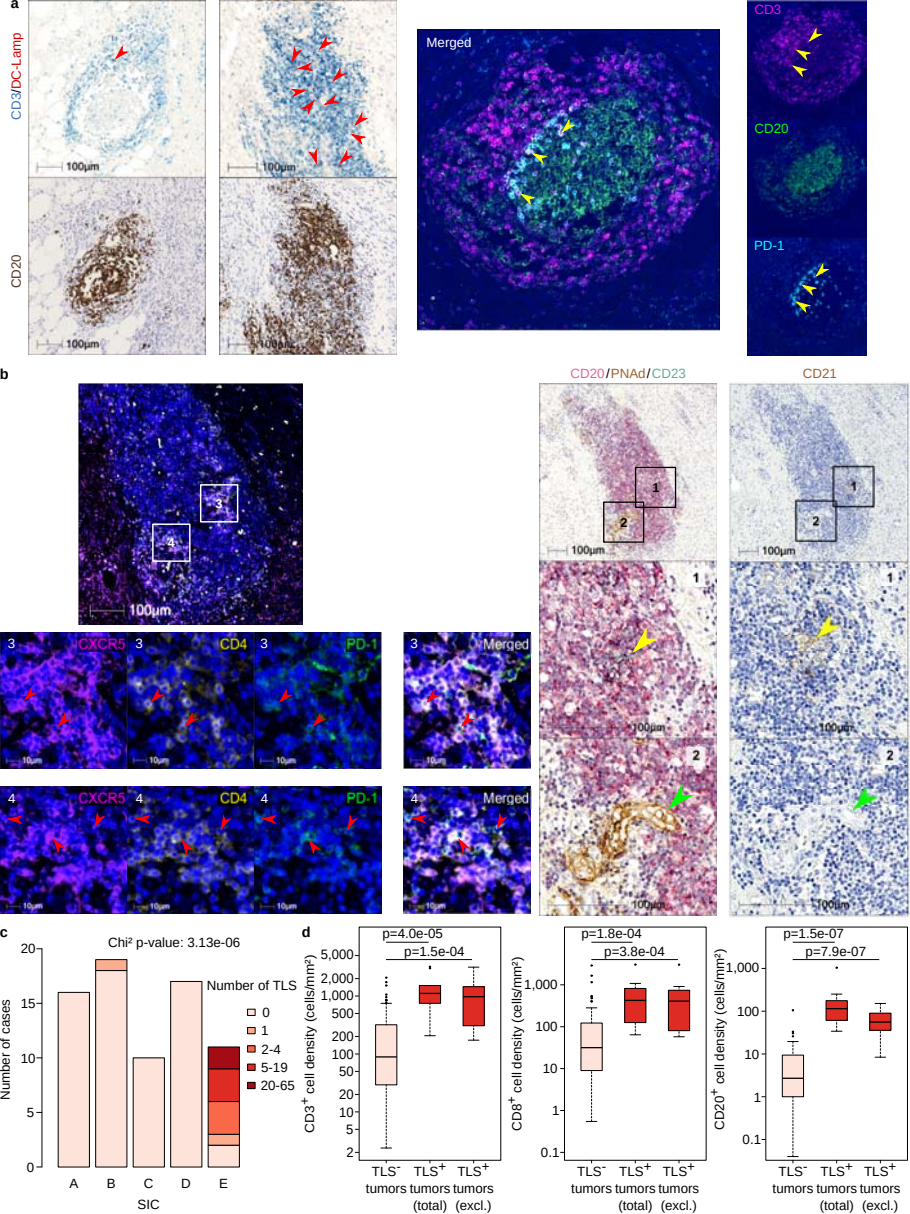


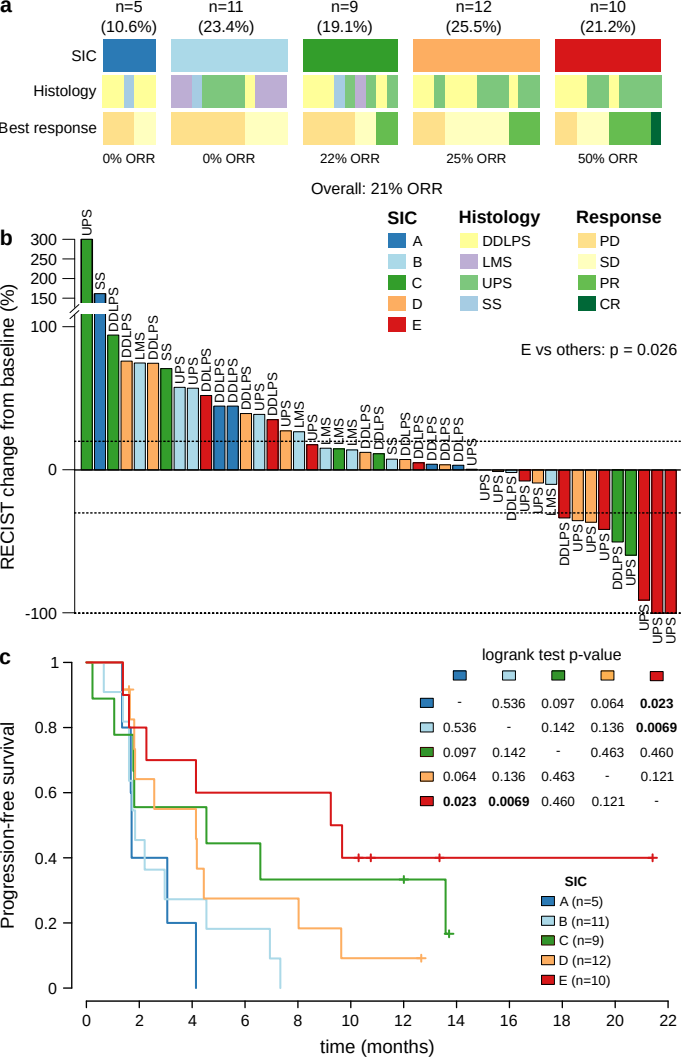
RESPONSE TO TREATMENT




Histology
DDLPS
LMS
UPS
SIC
A
B
C
D
E
n=55
(25.8%)
n=52
(24.4%)
n=35
(16.4%)
n=33
(15.5%)
n=38
(17.8%)
a
SIC
Histology
Adj.
p-value
b
T cells
1.5e-20
CD8+ T cells
7.7e-22
Cytotoxic lymphocytes
6.9e-27
NK cells
6.8e-21
B lineage
1.8e-29
Monocytic lineage
3.9e-20
Myeloid dendritic cells
8.0e-28
Neutrophils
1.9e-24
Endothelial cells
1.5e-20
c
Immunosuppression
8.7e-07
T cells activation
3.5e-21
T cells survival
2.2e-17
Regulatory T cells
3.5e-21
Class I MHC
6.5e-19
Myeloid cells chemotactism
7.1e-15
Tertiary lymphoid structures
1.4e-15
d
PD-1
8.4e-24
PD-L1
4.2e-06
PD-L2
2.2e-14
CTLA-4
1.4e-22
TIM3
5.3e-24
LAG3
4.1e-13
-4
0
4
Gene / Metagene
Z-score


a
Overall survival
logrank test p-value
- 0.408 0.438 0.048 0.025
0.408 - 0.922 0.225 0.130
0.438 0.922 - 0.304 0.192
0.048 0.225 0.304 - 0.711
0.025 0.130 0.192 0.711 -
time (months)
SIC
Number at risk
A 120 82 65 48 39 33
B 130 90 72 60 49 43
C 74 49 36 27 21 18
D 90 77 57 48 39 33
E 82 64 51 43 35 30
b
Variable
Hazard Ratio
p-value
SIC
A - -
B 0.683 0.27
C 1.05 0.88
D 0.373 0.011
E 0.418 0.029
FNCLCC grade
1 or 2 - -
3 1.46 0.15
Tumor size
Less than 10 cm - -
More than 10 cm 1.74 0.097
Histology
DDLPS - -
LMS 0.76 0.34
UPS 0.561 0.11
Depth
Deep - -
Superficial 0.452 0.099
Age
Less than median - -
More than median 2.04 0.0036
0.15 0.5 1.0 2.0 4.0
Hazard Ratio
c
Overall survival
logrank test p-value = 0.277
time (months)
CD8+ T cells
Number at risk
Hi 247 179 139 112 89 78
Lo 249 182 140 113 94 78
d
Overall survival
logrank test p-value = 4.25e-04
time (months)
B lineage
Number at risk
Hi 124 101 80 69 58 55
Lo 372 260 199 156 125 101
e
Overall survival
logrank test p-value
- 0.973 0.020 0.0033
0.973 - 0.047 0.011
0.020 0.047 - 0.487
0.0033 0.011 0.487 -
time (months)
B lineage / CD8+ T cells
Number at risk
Hi/Hi 82 62 48 41 32 30
Hi/Lo 42 39 32 28 26 25
Lo/Hi 165 117 91 71 57 48
Lo/Lo 207 143 108 85 68 53


a
CD3/DC-Lamp
CD20
100µm
Merged
CD3
CD20
PD-1
b
CD20/PNAd/CD23
CD21
1
2
3
4
100µm
CXCR5
CD4
PD-1
Merged
c
Chi² p-value: 3.13e-06
Number of cases
Number of TLS
0
1
2-4
5-19
20-65
A
B
C
D
E
SIC
d
p=4.0e-05
p=1.5e-04
CD3+ cell density (cells/mm²)
p=1.8e-04
p=3.8e-04
CD8+ cell density (cells/mm²)
p=1.5e-07
p=7.9e-07
CD20+ cell density (cells/mm²)
TLS- tumors
TLS+ tumors (total)
TLS+ tumors (excl.)


a
n=5 (10.6%)
n=11 (23.4%)
n=9 (19.1%)
n=12 (25.5%)
n=10 (21.2%)
SIC
Histology
Best response
0% ORR
0% ORR
22% ORR
25% ORR
50% ORR
Overall: 21% ORR
b
SIC
A
B
C
D
E
Histology
DDLPS
LMS
UPS
SS
Response
PD
SD
PR
CR
E vs others: p = 0.026
RECIST change from baseline (%)
300
250
200
150
100
0
-100
c
logrank test p-value
A
B
C
D
E
- 0.536 0.097 0.064 0.023
0.536 - 0.142 0.136 0.0069
0.097 0.142 - 0.463 0.460
0.064 0.136 0.463 - 0.121
0.023 0.0069 0.460 0.121 -
SIC
A (n=5)
B (n=11)
C (n=9)
D (n=12)
E (n=10)
Progression-free survival
1
0.8
0.6
0.4
0.2
0
0 2 4 6 8 10 12 14 16 18 20 22
time (months)