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Research article

# $CD20^{high}CD138^{low}$ tumor-infiltrating lymphocytes predominantly related to cytokine–cytokine receptor interactions are associated with favorable outcomes in neuroblastoma patients

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ABSTRACT

Recent advances have revealed that the role of the immune system is prominent in the antitumor response. In the present study, it is aimed to provide an expression profile of tumor-infiltrating lymphocytes (TILs), including mature B cells, plasma cells, and their clinical relevance in neuroblastoma. The expression of CD20 and CD138 was analyzed in the Cangelosi786 dataset (n = 769) as a training dataset and in our cohort (n = 120) as a validation cohort. CD20 high expression was positively associated with favorable overall survival (OS) and event-free survival (EFS) (OS: $P < 0.001$; EFS: $P < 0.001$) in the training dataset, whereas CD138 high expression was associated with poor OS and EFS (OS: $P < 0.001$; EFS: $P < 0.001$) in both the training and validation datasets. Accordingly, a combined pattern of CD20 and CD138 expression was

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developed, whereby neuroblastoma patients with $CD20^{high}CD138^{low}$ expression had a consistently favorable OS and EFS compared with those with $CD20^{low}CD138^{high}$ expression in both the training and validation cohorts ($P < 0.0001$ and $P < 0.01$, respectively). Examination of potential molecular functions revealed that signaling pathways, including cytokine–cytokine receptor interactions, chemokine, and the NF-kappa B signaling pathways, were involved. Differentially expressed genes, such as BMP7, IL7R, BIRC3, CCR7, CXCR5, CCL21, and CCL19, predominantly play important roles in predicting the survival of neuroblastoma patients. Our study proposes that a new combination of CD20 and CD138 signatures is associated with neuroblastoma patient survival. The related signaling pathways reflect the close associations among the number of TILs, cytokine abundance and patient outcomes and provide therapeutic insights into neuroblastoma.

## 1. Introduction

Neuroblastoma (NB), an embryonal tumor that originates from neural crest progenitor cells, occurs the sympathetic nervous system, with the most common location being the adrenal gland [1]. The evidences show that the 5-year overall survival (OS) rate of high-risk NB patients remains below 50 %, and the majority of patients develop disseminated relapsed disease, which highlights the additional therapies needed [1,2]. Immunotherapy has revolutionized cancer treatment and has mainly led to a significantly satisfying outcome in several adult solid cancers, and it also holds great promise for pediatric cancers [3]. The increased OS and event-free survival (EFS) of high-risk NB patients who follow the success of anti-disialoganglioside (GD2) therapy exemplifies the potential of immunotherapy for NB [4].

Therefore, a comprehensive understanding of tumor-infiltrating lymphocytes (TILs) is crucial for overcoming the challenges and opportunities associated with additional immunotherapy against NB. Several studies have shown that TIL subtypes include B and T cells, macrophages, and natural killer (NK) cells [5–7]. Tumor-infiltrating T cells are known to be involved in antitumor immunity, and cytotoxic $CD8^+$ T cells, which have been widely investigated in cancers, control tumor growth and metastasis [8]. In contrast, B lymphocytes and their expressed antibody signatures have only partially been identified. For instance, tertiary lymphoid structures (TLSs) that contain both T and B cells are observed in tumors and are linked to a potent immune response and favorable outcome, which suggests that B cells may cooperate with T cells in antitumor immunity [9]. On the contrary, B cells within TLSs are believed to trigger tumor cell tolerance, which is associated with poor prognosis [10]. Recent studies showed that $CD20^+$ B cells was to predict prognosis in patients with melanoma, breast and ovarian cancers [11–13].

Doki et al. demonstrated that TLS maturation is significantly associated with a great number of immune cells, particularly a remarkable increase in CD138 plasma cells, suggesting that CD138 expression used as an independent prognostic indicator in esophageal cancer [14]. Conversely, upregulated CD138 expression is associated with poor prognosis in breast cancer, probably because it interacts with αvβ3/5 integrins, which enhances angiogenesis [15].

However, the role of tumor-infiltrating B cells has not been as well studies, and in matters of prognostic importance, a consensus for NB has yet to be reached. To develop more efficient therapies for NB, it is crucial to figure out the role of B cells and plasma cells in the immune response.

Here, we present an extensive retrospective study on the role of tumor-infiltrating pan-B-cell antigen CD20, and CD138 on mature plasma cells using immunohistochemistry (IHC) combined with digital pathology. We also explore the prognostic roles of these markers and reveal potential targets for immunotherapy modalities.

## 2. Methods

### 2.1. Study population

Archived tumor tissues (n = 120) which served as a validation dataset were recruited between January 2016 and December 2020 at the Guangzhou Women and Children's Medical Center, Guangzhou Medical University. The Ethics Committee approved this project ([2021]078A01). Written informed consent was obtained from the parents/guardians of each patient in accordance with the Declaration of Helsinki. In addition, the external public dataset Cangelosi786 (n = 769) was included and used as a training dataset; the raw data are available in the original publication [16].

### 2.2. Antibodies used and immunohistochemistry

Pathological sequential slides with a thickness of 4 μm were sectioned from formalin-fixed, paraffin-embedded (FFPE) tumor tissues for IHC staining following the previous protocol [17–19]. Briefly, all tumor sections were sequentially followed with deparaffinization, rehydration, and then pre-incubation with hydrogen peroxide. These sections were then blocked with goat serum (Beyotime) for 10 min and incubated overnight at 4 °C with the primary antibodies CD20 (catalog#Kit-0001, clone L26; MXB, Fuzhou, China) and CD138 (catalog#MAB-0200, clone MI15; MXB, Fuzhou, China), respectively. A subsequent reaction was conducted with non-biotin HRP enzyme-labeled rabbit/mouse secondary antibody from an EliVision Super DAB detection system (MXB, Fuzhou, China) and finally counterstained with hematoxylin.

### 2.3. Density of CD20 and CD138

The density of positive staining for CD20 and CD138 was digitally quantified as the number of positive cells per square millimeter ($mm^2$), as described in detail in our previous studies [5,18]. Briefly, all pathological slides were scanned at 200x magnification (Pannaromic Scan 150, 3DHistech, Hungary) and autoexamined by digital analysis. Using Qupath (version 0.2.3, University of Edinburgh, Scotland), a pathologist manually circled the stained areas (K.C.). All nuclei in the tumor tissues were determined in hematoxylin channel image using a Mask-RCNN-based instance segmentation model [20]. Concretely, the positive nuclei were discerned based on the intensities in the DAB channel image, and then the density of the positive nuclei in the tumor tissues was obtained.

### 2.4. Cutoff values for high/low densities

All expression values of CD20 and CD138 with normalization were transformed into log2 values of z scores for further analysis [21]. The "Survminer" package in R (version 4.3.0) was conducted to obtain the optimal cutoff values that determine low and high densities of CD20 and CD138, respectively. Each patient was given a binary score, with 0 for low density and 1 for high density, for CD20 and CD138.

### 2.5. Functional analysis

To explore the biological characteristics of CD20 and CD138, a Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis was performed using the training dataset. We used the package *limma* (version 3.40.6) in R to identify differentially expressed genes (DEGs) between the $CD20^{low}CD138^{high}$ and $CD20^{high}CD138^{low}$ groups. Significant DEGs were determined using the following parameters: a fold change (FC) greater than 1.2 times and a corrected *P* value less than 0.05. The package *clusterProfiler* (version 3.17) in R was then used to show the KEGG results.

### 2.6. Statistical analysis

The primary outcome of interest was OS (time from cancer diagnosis to death due to any cause or the last follow-up). Additional outcome was to evaluate the EFS for time to tumor progression, defined as time to first observation of disease recurrence or the last follow-up.

Correlations between $CD20^+$ and CD138+ TILs and clinical features were performed using a $\chi 2$ test or Fisher's exact test. We did the correlation between survival time and expressions of CD20 and/or CD138 or the combined CD20 and CD138 signature using Kaplan–Meier method. The statistical significance was defined using the Log-rank test. Univariate and multivariant Cox proportional hazards model were used for analysis of hazard ratios (HRs) with 95 % confidence intervals (CIs). Only variables for which $P < 0.05$ in the univariate analysis were incorporated into the multivariable Cox regression analysis. All testing was carried out using R (version

**Table 1**
Clinicopathological characteristics of NB patients from two cohorts in this study.

| Characteristics | | Training cohort | Validation cohort | *P* value |
|---|---|---|---|---|
| | | Cangelosi786 (n, %) | Our dataset (n, %) | |
| **Total** | | 769 | 120 | |
| **INSS** | | | | 0.505 |
| | Early | 353 (45.9 %) | 50 (41.7 %) | |
| | Advanced | 415 (54.0 %) | 70 (58.3 %) | |
| | Unknown | 1 (0.1 %) | 0 | |
| **Age, month** | | | | 0.818 |
| | ≥18 | 329 (42.8 %) | 50 (41.7 %) | |
| | <18 | 440 (57.2 %) | 70 (58.3 %) | |
| **MYCN** | | | | **0.004** |
| | Nonamp | 614 (79.8 %) | 111 (92.5 %) | |
| | Amp | 151 (19.6 %) | 9 (7.5 %) | |
| | Unknown | 4 (0.6 %) | 0 | |
| **INPC** | | | | **<0.001** |
| | FH | 591 (76.9 %) | 54 (45.0 %) | |
| | UH | 178 (23.1 %) | 66 (55.0 %) | |
| **Event overall** | | | | 0.148 |
| | no | 547 (71.1 %) | 93 (77.5 %) | |
| | yes | 222 (28.9 %) | 27 (22.5 %) | |
| **Event-free** | | | | 0.739 |
| | no | 449 (58.4 %) | 72 (60.0 %) | |
| | yes | 320 (41.6 %) | 48 (40.0 %) | |
| **Follow-up duration (days), median (IQR)** | | 2495 (1322, 3626) | 1444 (1015, 2036) | **<0.001** |

**Abbreviations:** NB, neuroblastoma; INSS, the International Neuroblastoma Staging System; INPC, the International Neuroblastoma Pathology Classification; FH, favorable histology; UH, unfavorable histology; IQR, interquartile range. Statistically significant *P* values are shown in bold.

4.3.0) and Stata version 15.1 (Texas, USA). The two-sided *P* value less than 0.05 was defined as statistically significant.

## 3. Results

### 3.1. Patient

Two independent cohorts were included in this study. Table 1 lists the demographics and clinical features of all the NB patients. Specifically, the NB patients in our cohort were followed up until August 31, 2023. The median follow-up time was 1444 days (interquartile range [IQR] 1015–2036) in our cohort and 2495 days (IQR 1322-3626) in the Cangelosi786 dataset, respectively. The survival estimates were similar in the two datasets. The 3-year and 5-year OS rates were 77 % (74%–80 %) and 72 % (69%–75 %) in the training cohort, and 81 % (73%–87 %) and 74 % (64%–81 %) in the validation cohort, respectively.

### 3.2. Association of CD20 and CD138 expression with survival

To assess the associations between CD20 and CD138 expression and survival in NB, we performed IHC experiment of FFPE tissue samples. IHC images of positive controls from lymph nodes and representative images of IHC staining from NB tissue samples are shown in Fig. 1A–B and Fig. 1C–F, respectively. We quantified gene expression within the specified areas using digital pathology (Supplementary Figure S1), and the values are presented as medians with ranges, where the number of positive staining events was 111/mm$^2$ (6-2309) for CD20 and 6/mm$^2$ (1–516) for CD138. By integrating with the gene expression data from the training dataset (data not shown), we found that the overall expression of CD20 and CD138 was relatively low in NB patients. In addition, we analyzed the correlation between CD20 and CD138 and detected very weak or no correlation between the expression of CD20 and that of CD138 in the training and validation datasets (Supplementary Figure S2).

As a result of the low expression of CD20 and CD138 in NB, we first sought to determine the importance of their potential roles and used a public dataset as a training cohort to estimate survival outcomes in NB patients with different expression levels of CD20 and CD138, respectively. Interestingly, high expression of CD20 was associated with good OS and EFS (**OS***: $P < 0.001$, Fig. 2A; **EFS:** $P < 0.001$, Supplementary Figure S3A), whereas high expression of CD138 was associated with poor OS and EFS (**OS***: $P < 0.001$, Fig. 2C; **EFS:** $P < 0.001$, Supplementary Figure S3C). In the validation cohort, we obtained concordant results for CD138 only (**OS:** $P = 0.002$, Fig. 2D; EFS: $P < 0.001$, Supplementary Figure S3D), not for CD20 (**OS:** $P > 0.05$, Fig. 2B; EFS: $P > 0.05$, Supplementary Figure S3B). These findings suggest the importance of CD20 or CD138 in the prognosis of patients with NB and highlight the limitations of a single marker in defining the prognosis of these patients.

### 3.3. Prognostic value of the integrated pattern of CD20 and CD138 expression

To further comprehend the prognostic value of CD20 and CD138 expression, we used the combination of CD20 and CD138 to group patient samples into four subtypes according to gene expression: CD20$^{low}$CD138$^{low}$, CD20$^{high}$CD138$^{low}$, CD20$^{low}$CD138$^{high}$, and CD20$^{high}$CD138$^{high}$. Surprisingly, the overall difference in OS and EFS estimates was significant (Fig. 3). Specifically, we found that CD20$^{high}$CD138$^{low}$ NB patients had consistently favorable OS and EFS compared with CD20$^{low}$CD138$^{high}$ NB patients in the training dataset ($P < 0.0001$, Fig. 3A; $P < 0.0001$, Fig. 3B). A similar finding between these two groups was observed in the validation cohort (*P*

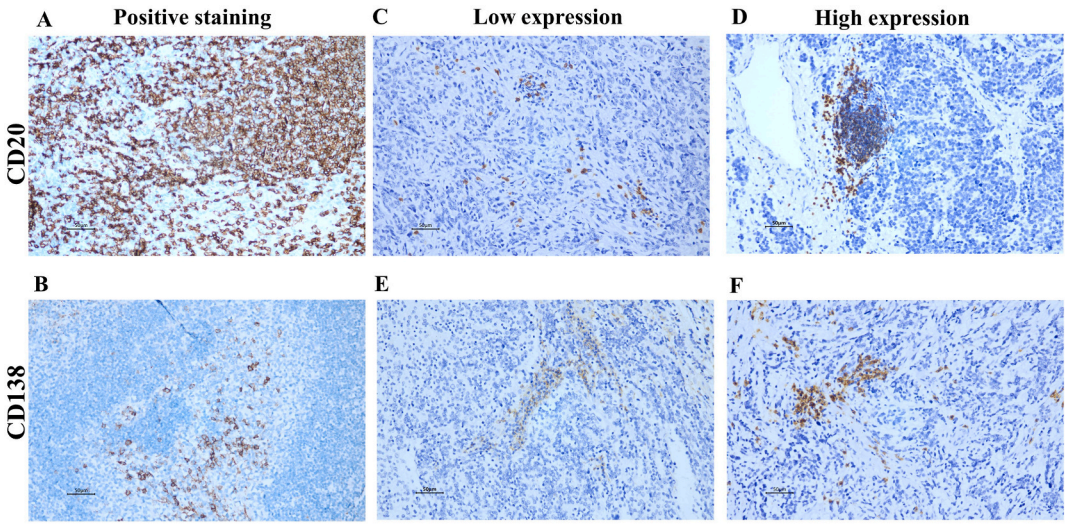


**Fig. 1.** The IHC images for positive controls of CD20 (A) and CD138 expression (B) and representative images of CD20 (C–D) and CD138 (E–F) expression in the validation cohort.

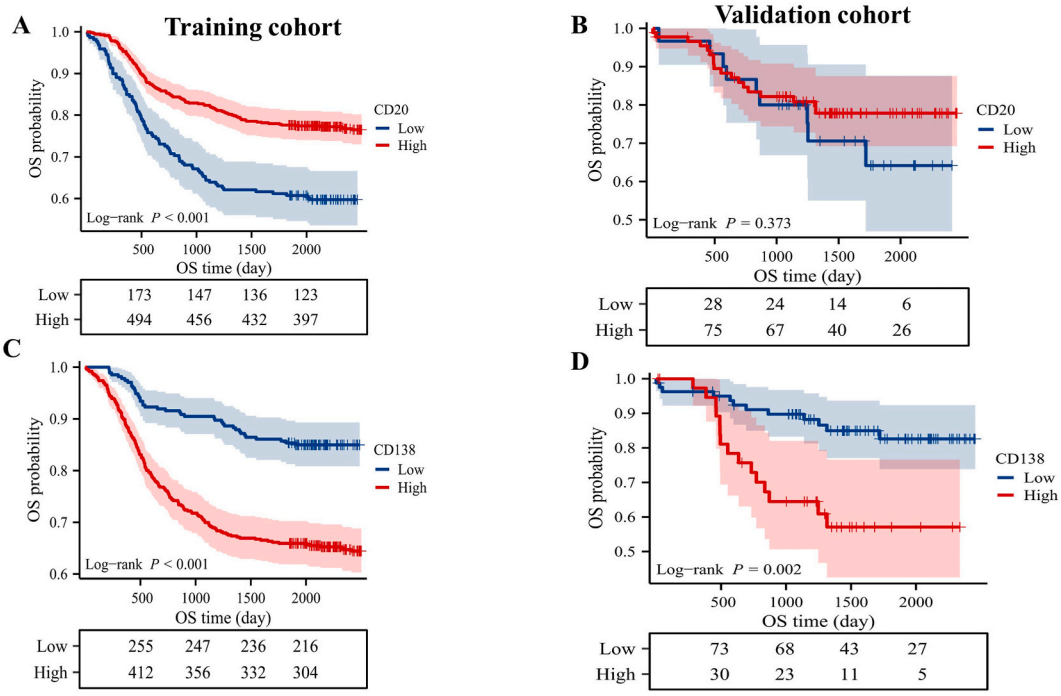


**Fig. 2.** Overall survival analysis of NB patients stratified by CD20 and CD138 expression in in the training and validation datasets. Survival curves were generated according to high and low expression of CD20 and CD138 (**CD20**: A and B; **CD138:** C and D, respectively). *P* values were calculated using the log-rank test.

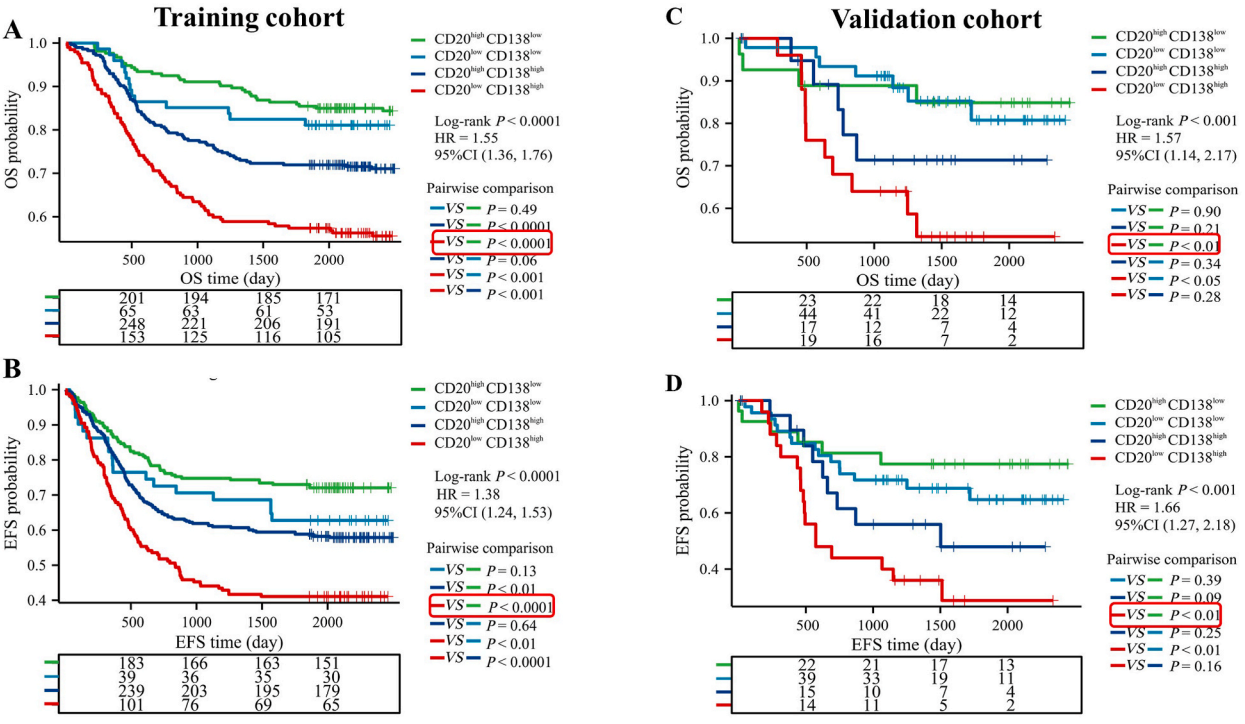


**Fig. 3.** Survival analysis of combined CD20 and CD138 expression in NB patients in the training and validation datasets. Kaplan–Meier survival curves were generated for the four groups (**OS**: A and C; **EFS**: B and D, respectively). *P* values were calculated using the log-rank test.

$< 0.01$, Fig. 3C; $P < 0.01$, Fig. 3D). The detailed 3- and 5-year survival estimates are listed in Supplementary Table S1.

We next investigated the prognostic value of the combination of the CD20 and CD138 signatures after adjusting for clinical variables. Specifically, we constructed a multivariate Cox hazards regression model using the training dataset that contained significant factors based on the univariate Cox regression analysis (Supplementary Table S2). As shown in Table 2, the combination of $CD20^{low}CD138^{high}$ expression remained significant for predicting patient survival even after clinical variables were considered (**OS:** HR = 1.61, 95 % CI = 1.02–2.55, $P = 0.042$; **EFS:** HR = 1.79, 95 % CI = 1.26–2.55, $P = 0.001$). We did not observe a similar result for the validation cohort, which was presumably due to the relatively small sample size (n = 52).

Taken together, these results demonstrate that the expression of CD20 and CD138 is inversely correlated to some degree and has a significant effect on NB patient survival.

### 3.4. Predicting functions and pathways

To reveal the biological functions of the CD20/CD138 signature for NB prognosis, we reviewed all DEGs between the $CD20^{low}CD138^{high}$ and $CD20^{high}CD138^{low}$ patient groups; the top 50 DEGs are displayed in Fig. 4A. According to this analysis, signaling pathways, including cytokine–cytokine receptor interactions, chemokine, and the NF-kappa B pathways as well as the corresponding DEGs, such as BMP7, IL7R, BIRC3, CCR7, CXCR5, CCL21, and CCL19, predominantly played important roles in predicting the survival of NB patients (Fig. 4B and C).

In addition to the DEGs and pathways indicated above, there has been growing interest in utilizing specific components of the ubiquitin-proteasome system as drug targets to treat cancer [22,23]. Interestingly, a series of deubiquitylating enzyme (DUB) changes was found between the two groups, including changes in genes such as USP46, USP48, USP24, USP18, USP53, ATXN3, PAN2, MYSM1, and TNFAIP3.

In summary, these analyses revealed numerous DEGs related to tumorigenesis as well as potential targets in NB, which may contribute to novel prognostic and therapeutic regimens in the future.

## 4. Discussion

The study is to simultaneously assess CD20 expression on mature B-cells and CD138 expression on plasma cells along with their prognostic role in NB patients and the principal finding was that NB patients with the $CD20^{high}CD138^{low}$ signature had better survival outcomes than those with the $CD20^{low}CD138^{high}$ signature. We also preliminarily explored the underlying mechanisms for survival difference between the two groups. Our study elucidates the impact of the expressions of CD20 and CD138 on NB patient outcomes and reveals biomarkers and therapeutic targets.

In this study, we used the digital pathology, a common-used tool for automatic quantification of full-view slides, which was performed in our previous study to quantify the expressions of CD20 and CD138. We analyzed the expression of these markers in the training and validation datasets and detected low expression in NB patients. This finding is similar to findings of previous studies that reported few of infiltrating B cells in NB [24,25]. This is presumably because these cases of NB were immunologically 'cold' tumors [26]. Nevertheless, CD20 and CD138 remain targets for prognosis and therapeutic regimens.

As expected, $CD20^{high}$ expression was associated with good OS and EFS only for patients in the training dataset; however, $CD138^{high}$ expression was associated with poor OS and EFS of patients in both the training and validation datasets. Although Chen et al. reported that high levels of infiltrating $CD20^{+}$ naïve and memory B cells are associated with both recurrence-free survival and overall survival in NB patients, the roles of CD138 have not yet been reported [27]. In addition, high expression of CD20 has been associated with better survival in patients with hepatocellular [28] and ovarian cancers [29]. On the contrary, a high level of the

**Table 2**
Multivariate Cox hazard regression analysis of factors associated with survival in NB patients from two datasets, respectively.

| | Training cohort (n = 410) | | Validation cohort (n = 52) | |
|---|---|---|---|---|
| Factors | HR (95 % CI) | P value | HR (95 % CI) | P value |
| **OS** | | | | |
| **INSS** (Advanced *vs.* early) | 3.73 (1.97–7.08) | **< 0.001** | NA | NA |
| **Age, months** (≥18 *vs.* <18) | 2.37 (1.49–3.78) | **< 0.001** | 0.28 (0.07–1.18) | 0.082 |
| **MYCN** (Amp *vs.* nonamp) | 2.58 (1.59–4.18) | **< 0.001** | 4.04 (0.92–17.68) | 0.064 |
| **INPC** (UH *vs.* FH) | 1.33 (0.84–2.11) | 0.228 | 0.77 (0.23–2.64) | 0.679 |
| **Combined** ($CD20^{low}CD138^{high}$ *vs.*$CD20^{high}CD138^{low}$) | 1.61 (1.02–2.55) | **0.042** | 0.47 (0.12–1.82) | 0.272 |
| **EFS** | | | | |
| **INSS** (Advanced *vs.* early) | 1.67 (1.12–2.49) | **0.011** | NA | NA |
| **Age, months** (≥18 *vs.* <18) | 1.63 (1.15–2.31) | **0.006** | 0.45 (0.13–1.52) | 0.197 |
| **MYCN** (Amp *vs.* nonamp) | 1.36 (0.89–2.09) | 0.157 | 1.40 (0.36–5.37) | 0.627 |
| **INPC** (UH *vs.* FH) | 1.60 (1.06–2.42) | **0.024** | 1.52 (0.53–4.33) | 0.432 |
| **Combined** ($CD20^{low}CD138^{high}$ *vs.*$CD20^{high}CD138^{low}$) | 1.79 (1.26–2.55) | **0.001** | 0.69 (0.22–2.17) | 0.524 |

**Abbreviations**: NB, neuroblastoma; INSS, the International Neuroblastoma Staging System; INPC, the International Neuroblastoma Pathology Classification; FH, favorable histology; UH, unfavorable histology; OS, overall survival; EFS, event-free survival; HR, hazard ratio; CI, confidence interval; NA, not available. Statistically significant *P* values are shown in bold.

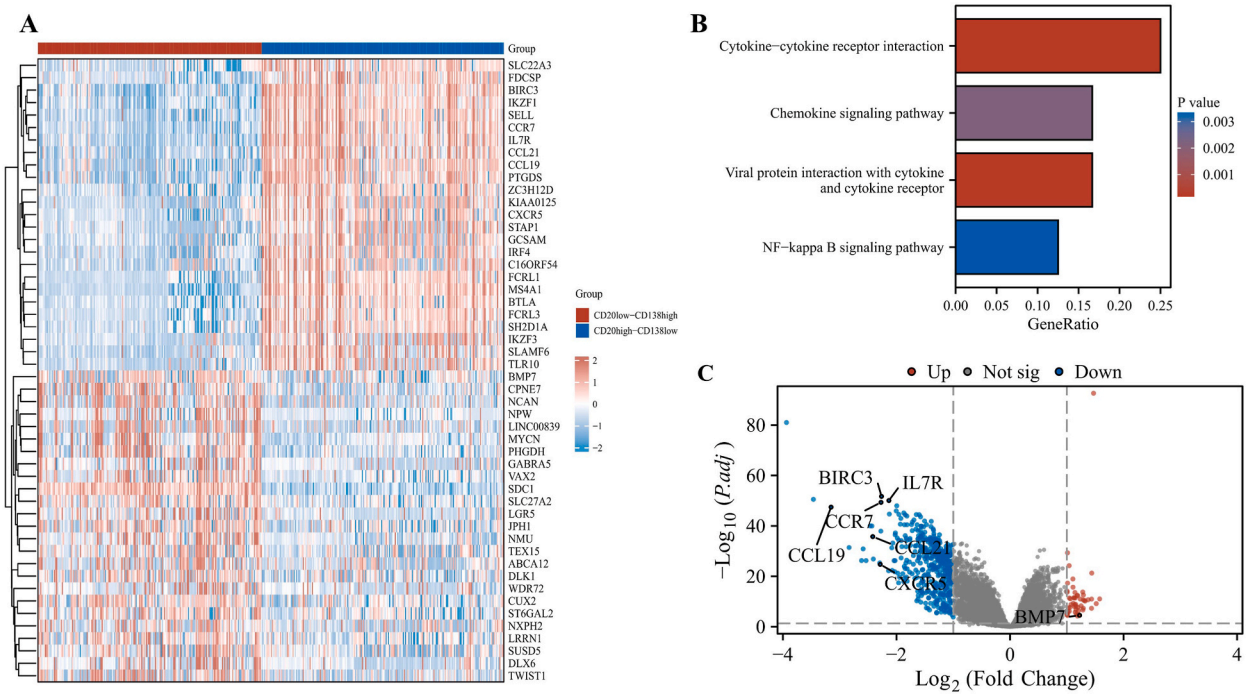


**Fig. 4.** Biological function analysis of DEGs in the training dataset. Heatmap showing the top 50 DEGs between the $CD20^{low}CD138^{high}$ and $CD20^{high}CD138^{low}$ groups (A); a histogram showing the results of KEGG enrichment analysis based on these top 50 DEGs (B); a volcano plot displaying DEGs based on the KEGG results (C).

plasma cell marker CD138 indicates a high proliferation rate in myeloma cells and enhances IL6R signaling, which is known to promote tumor development [30]. In solid tumors such as ovarian adenocarcinomas, stromal expression of CD138 has been confirmed as a poor prognostic factor [31,32]. Nevertheless, another investigation recently reported a contradictory result, in which cancer patients with brain metastasis who had high expression of CD138 remained alive for a longer time than patients with low CD138 expression [33]. It seems that CD138 expression is a favorable biomarker in certain cancer types, but such a discrepancy may reflect different biological behaviors and not only different cancer types.

In contrast to a single gene, such as CD20 or CD138, we discovered that the combination of CD20 and CD138, especially $CD20^{high}CD138^{low}$ and $CD20^{low}CD138^{high}$, had a significant effect on OS and EFS. Similarly, a previous study reported that a high ratio of $CD20^{+}$ B cells/CD138+ plasma cells is associated with a tendency toward longer survival in patients with colorectal cancer [34]. Based on these observations, we concluded a possible inverse correlation between the densities of CD20 and CD138 expression, which agreed with recent investigations. Several studies have shown that memory B cells that express CD19 in peripheral lymphoid organs are activated by antigens and differentiate into short-lived proliferating plasma blasts with $CD19^{+}$, $CD20^{low}$, and $CD138^{+}$ phenotypes. These cells then migrate into the bone marrow or the lamina propria of the mucosa, where they become plasma cells and secrete abundant immunoglobulins [35,36]. Furthermore, a previous study showed a connection in patients with ulcerative colitis in the remission stage, in which proliferative $CD20^{low}$ and $CD138^{+}$ cells suggested the proliferation and differentiation of memory B cells into plasma cells [37]. Therefore, our findings indicate that $CD138^{high}$ NB patients with or without low CD20 expression exhibit a pattern of mature plasma cells. In summary, such reverse recruitment of B cells and plasma cells, which was first reported in NB, occurs in patients and plays an important role in tumorigenic potential, which requires further investigation in the future.

Our finding that B-cell abundance is associated with survival is in line with the findings of another study [27] in which B-cell abundance in relation to a hot tumor microenvironment was proposed for NB and indicated an effective antitumor immune response [38,39]. Therefore, we compared DEGs between the $CD20^{high}CD138^{low}$ and $CD20^{low}CD138^{high}$ groups and observed potential biological functions in NB. Specifically, the cytokine−cytokine receptor interaction pathway, which included the genes CCL19/CCL21/CXCR5/CCR7/IL7R/BMP7, was strongly enriched. Cytokines are recognized as comprehensive immunoregulatory molecules critical for tumor progression and might be used as immune-related therapeutic targets [40]. For instance, some reports have shown that CCR7 and CXCR5 are expressed in cancer cells and that the CCL19/CCL21/CCR7 axis and CXCL13/CXCR5 axis play crucial roles in activating the immune response [41–43]. In addition, we found that several DUBs were differentially expressed in NB. Accumulating evidence indicates that DUBs modulate immune-oncology [44]; clinical trials of their inhibitors are planned and will contribute to the current interest in DUBs as drug targets [45,46]. Given these properties, the antitumor immune response of DUBs in the TME is of great interest, as this mode of therapy provides a new opportunity for NB treatment. However, further studies are required to clarify the exact mechanisms involved of CD20/CD138 expression affecting the indicated pathways.

Although our study provides important insights into $CD20^{+}$ and CD138+ TILs in NB, a few limitations should be noted. Survival

outcomes were limited because the relatively small size of samples was recruited in the validation dataset. Although we revealed significant signaling pathways, and further refinement of the experimental design and interpretation of the results are warranted, which might provide insight for therapeutic development in NB.

In the study, we profiled a CD20 and CD138 signature that may predict survival outcomes. The related signaling pathways and DEGs reflect the associations among the levels of TILs, cytokine abundances, and NB patient outcomes and provide therapeutic insights into NB.

## Ethics statement

The Ethics Committee of Guangzhou Women and Children's Medical Center, Guangzhou Medical University approved this project in accordance with the Declaration of Helsinki ([2021]078A01). The parents/guardians of each patient provided written informed consent before the study began.

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## Data availability statement

The data reported in the study is included within the article and its supplementary files or available from the corresponding author upon reasonable request.

## CRediT authorship contribution statement

**Liang-Jun Qin:** Writing – original draft, Resources, Methodology. **Hui Xu:** Writing – original draft, Methodology, Formal analysis. **Li-Ping Li:** Methodology, Data curation. **Shu-Hua Li:** Software, Data curation. **Shuo-Yu Xu:** Software, Formal analysis. **Kai Chen:** Software, Formal analysis. **Tianyou Yang:** Methodology, Data curation. **Feng-Hua Wang:** Methodology, Data curation. **Liandong Zuo:** Resources, Data curation. **Liang Zeng:** Writing – original draft, Project administration, Investigation. **Hai-Yun Wang:** Writing – review & editing, Writing – original draft, Project administration, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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## Appendix A. Supplementary data

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