Checkpoint receptors TIGIT and PD-1 are co-expressed in T cells from B-cell non-Hodgkin lymphoma tumors and correlate with reduced T-cell effector function

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RUNNING TITLE

TIGIT and PD-1, and their cognate ligands, in non-Hodgkin lymphoma

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CONFLICT OF INTEREST DISCLOSURE

All authors declare no competing financial interests.

ABSTRACT

Checkpoint blockade can reverse T-cell exhaustion and promote antitumor responses. While blocking the PD-1 pathway has been successful in Hodgkin lymphoma, response rates have been modest in non-Hodgkin lymphoma (NHL). Co-blockade of checkpoint receptors may therefore be necessary to fully unleash antitumor T-cell responses, and investigation of co-inhibitory receptor expression is warranted to determine relevant targets. Here, in-depth characterization of co-inhibitory receptor expression in intratumoral T cells from different NHL types identified TIGIT and PD-1 as the most frequently expressed co-inhibitory receptors. NHL tumors were enriched in CD8 and CD4 T_{EM} cells that displayed remarkably high co-expression of TIGIT and PD-1. Importantly, TIGIT and PD-1 expression correlated with reduced production of IFN-γ, TNF-α and IL-2. The suppressed cytokine production could be improved upon *in vitro* culture in absence of ligands. While PD-L1 was expressed by macrophages, the TIGIT ligands CD155 and CD112 were expressed by lymphoma cells in 39% and 50% of DLBCL cases and in some MCL cases, and by endothelium and FDCs in all NHL investigated. Collectively, our results show that TIGIT and PD-1 mark dysfunctional T cells and suggest that TIGIT and PD-1 co-blockade should be further explored to elicit potent antitumor responses in patients with NHL.

INTRODUCTION

T-cell activation is initiated upon antigen recognition by the T-cell receptor (TCR) and is further potentiated by activation of co-stimulatory receptors (1). This is counteracted by co-inhibitory receptors such as CTLA-4 and PD-1, which are transiently induced upon TCR activation to balance acute immune responses. In chronic infection and cancer, high expression of co-inhibitory receptors persists, leading to impaired T-cell function (2). Consequently, immune checkpoint blockade targeting co-inhibitory receptors such as PD-1 has emerged as a promising immunotherapeutic approach (3). However, while PD-1/PD-L1 blockade as monotherapy has been highly successful in relapsed/refractory (R/R) Hodgkin lymphoma with objective response rates (ORR) of 65-87% (4-7), the benefits have overall been modest in non-Hodgkin lymphoma (NHL), demonstrated by ORR of 40% in R/R follicular lymphoma (FL) (8), 36% in R/R diffuse large B-cell lymphoma (DLBCL) (8) and no responders in relapsed chronic lymphocytic leukemia (CLL) (9), despite frequent expression of PD-1 and PD-L1 (10). Reliable data on PD-1 blockade in mantle cell lymphoma (MCL) is currently lacking. As progression of T-cell exhaustion is linked to expression of increased number of co-inhibitory receptors (2), checkpoint co-blockade may be necessary to achieve optimal antitumor T-cell responses. However, while PD-1 expression has been well studied in NHL (10), expression of other immune checkpoint receptors is less characterized. A thorough investigation of co-inhibitory receptor expression patterns is warranted to determine relevant targets for checkpoint blockade.

TIGIT (T-cell immunoglobulin and ITIM domain) is a newly described co-inhibitory receptor that can be expressed by effector T cells, natural killer (NK) cells, T regulatory cells (Tregs) and T follicular helper (T_{FH}) cells (11-14). TIGIT has recently gained attention as a new therapeutic target in cancer due to its frequent expression on tumor-infiltrating T cells, and TIGIT expression is associated with CD8 T-cell exhaustion (15-17). The TIGIT ligands, CD155 and CD112, can be expressed by different cell types, including antigen-presenting cells and tumor cells (11,18,19). We recently identified TIGIT as a potential target for checkpoint blockade in FL by demonstrating that intratumoral CD8 T cells with dysfunctional TCR signaling were identified by TIGIT expression (20). However, the role of TIGIT in other NHLs has not been addressed. Here, multicolor flow cytometry was used to characterize the landscape of co-inhibitory receptor expression in distinct T-cell subsets from DLBCL, MCL, FL, CLL and marginal zone lymphoma (MZL). Our aim was to identify the most relevant

checkpoint receptors for clinical investigation as targets for checkpoint blockade in NHL. We examined the numbers of TIGIT and PD-1 positive intratumoral T cells, correlated TIGIT and PD-1 expression with the T cells' capacity to produce cytokines, and also report expression of the TIGIT and PD-1 ligands in the tumor microenvironment.

MATERIALS AND METHODS

Patient samples

Samples were obtained with informed consent in accordance with the Declaration of Helsinki and with approval from the Regional Committee for Medical and Health Research Ethics. Tumor biopsies were obtained from patients with FL (*n* = 19), DLBCL (*n* = 19), MCL (*n* = 11), CLL (*n* = 7) and MZL (*n* = 2) at the Norwegian Radium Hospital, Oslo, Norway; clinical characteristics described in Supplementary Table S1. DLBCL samples included non-GCB (*n* = 15) and GCB (*n* = 4) subtypes. Tonsils were obtained from patients (*n* = 19) undergoing tonsillectomy at Agroklinikken (Asker, Norway). Samples were processed to single cell suspensions by mincing and cryopreserved in liquid nitrogen.

Flow cytometry

Flow cytometry analysis was performed as previously described (20). Single cells were stained with the following antibodies: CD3-Pacific Blue (clone UCHT1), CCR7-PE (150503), CXCR5-Ax488 (RF8B2), CD20-APCH7 (L27), PDL1-APC (MIH1), PDL2-APC (MIH18) and IFNγ-PE (4S.B3) from BD Biosciences, TIGIT-APC (MBSA43), LAG3-PeCy7 (3DS223H), TNFα-Ax488 (MAb11) and IL2-PeCy7 (MQ1-17H12) from eBioscience, and CD4-Ax700 (RPA-T4), CD8-Bv785 (RPA-T8), CD45RA-Bv510 (HI100), PD1-Bv650 (EH12.2H7), TIM3-APC (F38-2E2), BTLA-APC (MIH26), CD244-PerCPCy5.5 (C1.7), CD160-PeCy7 (BY55), LAIR1-PerCPCy5.5 (NKTA255), CD155-PE (SKII.4) and CD112-PeCy7 (TX31) from Biolegend. Brilliant Stain Buffer (BD biosciences) was used as staining buffer. Data was acquired on LSR II (BD Biosciences) and analyzed using Cytobank (https://www.cytobank.org/).

Immunohistochemistry

Serial sections of cryopreserved tissue were stained with antibodies for CD155 (L95) and CD112 (L14) as previously described (20), in addition to PD-L1 (405.9A11) and CD68 (KP1).

Analysis of cytokine production

T cells from NHL tumors were enriched by depletion of $CD19^+$ B cells using Dynabeads™ CD19 (ThermoFisher) according to the manufacturer's protocol. Cytokine production was then activated for 6 hours using Dynabeads™ Human T-Activator CD3/CD28 (ThermoFisher) in a 1:1 bead-to-cell ratio, with GolgiPlug (BD Biosciences) present for the last 4 hours. Cells were fixed in paraformaldehyde (PFA; 1.6%) to stop activity, followed by centrifugation and permeabilization in >90% ice-cold methanol. Samples were stored at -80°C before staining with antibodies and flow cytometry acquisition.

RESULTS AND DISCUSSION

TIGIT and PD-1 are highly expressed in intratumoral T cells

Characterization of co-inhibitory receptor expression revealed that TIGIT and PD-1 were expressed at higher frequency than all other receptors investigated (Fig. 1A). Strikingly, TIGIT was expressed at increased frequency on T cells from NHL tumors as compared to tonsillar T cells from healthy donors (Fig. 1B). Although not statistically significant, a similar trend was observed for PD-1 expression (Fig. 1B). This was consistent with previous reports from gene expression analysis, showing upregulation of TIGIT and PD-1 in FL and DLBCL as compared to normal controls (21). In FL, high expression of both TIGIT and PD-1 in CD4 T cells correlated with advanced disease stage (Supplementary Fig. S1). Our results further showed that TIGIT and PD-1 surface expression varied strongly between T-cell subsets. While few naïve T cells expressed TIGIT or PD-1, the vast majority of CD8 and CD4 T effector memory (T_{EM}) cells were positive for the two co-inhibitory receptors (Supplementary Fig. S2-S3; Fig. 2A-B). This is important as T-cell distribution was strongly skewed towards T_{EM} cells, the main subset among CD8 as well as CD4 intratumoral T cells across all NHLs investigated (Supplementary Fig. S4A-B). Remarkably, tumor-infiltrating CD8 and CD4 T_{EM} cells had TIGIT median expression ranging from 83-95% and 85-93%, respectively (Fig. 2A-B). PD-1 median expression ranged from 81-85% in CD8 T_{EM} cells, and 70-75% in CD4 T_{EM} cells (Fig. 2A-B). Interestingly, CD8 T_{EM} cells in NHL, independent on type of lymphoma, expressed TIGIT and PD-1 at significantly increased frequencies compared to tonsillar T cells. The percentage of $TIGIT^+$ cells was also significantly increased in NHL CD4 T_{EM} cells as compared to the tonsillar counterpart (Fig. 2A-B). In contrast, CD244 was expressed at lower

and variable frequencies among CD8 T_{EM} cells, also within the same type of NHL, while BTLA, TIM-3, LAG-3, CD160 and LAIR-1 were expressed by in average <20% of CD8 and CD4 T_{EM} cells (Fig. 2A-B, Supplementary Figure S2-S3).

TIGIT and PD-1 are co-expressed in intratumoral T cells

As co-expression of TIGIT and PD-1 has been associated with T-cell dysfunction in cancer (15) we next investigated the level of co-expression of these two receptors in NHL (Fig. 2C). Strikingly, TIGIT and PD-1 were co-expressed by the vast majority of T_{EM} cells. In average 78-83% of CD8 T_{EM} cells and 69-79% of CD4 T_{EM} cells co-expressed the two co-inhibitory receptors, across the different NHLs (Fig. 2D). A significantly higher degree of co-expression was observed in CD8 T_{EM} cells from all lymphoma types investigated as compared to the tonsillar counterpart (Fig. 2D). For CD4 T_{EM} cells, TIGIT and PD-1 co-expression was significantly increased in FL, CLL and MZL (Fig. 2D).

To benefit from checkpoint blockade, patients must previously have developed tumor-specific T cells. Hence, if the population of intratumoral $TIGIT^{+}PD\text{-}1^{+}$ T_{EM} cells found in NHL is enriched for exhausted, neoantigen-experienced T cells, targeting these receptors by checkpoint blockade might be efficient in restoring antitumor reactivity. Support for this hypothesis comes from emerging evidence that tumor neoantigens can be expressed in NHL. In MCL, neoantigenic mutations presented by MHC were exclusively derived from the lymphoma immunoglobulins, and could induce T-cell responses (22). Furthermore, analysis of mutations, transcriptional profiles and single-cell TCR sequences in melanoma tumor samples obtained before and during treatment, revealed expansion of T-cell clones and putative selection against neoantigenic mutations in patients who responded to PD-1 blockade by nivolumab (23).

TIGIT and PD-1 correlate with reversible suppression of cytokine production

We hypothesized that intratumoral T-cell function correlated with expression of TIGIT and PD-1. To test this, cytokine production was measured in relation to TIGIT and PD-1 expression in CD4 and CD8 T cells from NHL. Strikingly, T cells expressing either TIGIT or PD-1 produced low levels of IFN-γ, TNF-α and IL-2 compared to cells negative for the receptors (Fig. 3A). Among CD8 T cells, intracellular TNF-α and IL-2 was significantly reduced in $PD\text{-}1^{+}TIGIT^{+}$ and $PD\text{-}1^{+}TIGIT^{-}$ cells compared to $PD\text{-}1^{-}TIGIT^{-}$ cells, and the same trend was observed for IFN-γ (Fig. 3B). Among CD4 T cells, the capacity to produce TNF-α

and IL-2 was significantly reduced in PD-1^{+}TIGIT^{+} cells as compared to PD-1^{-}TIGIT^{-} or PD-1^{+}TIGIT^{-} cells, while IFN-γ production was lower in PD-1^{+} TIGIT^{+} and PD-1^{-}TIGIT^{+} cells as compared to PD-1^{+}TIGIT^{-} cells (Fig. 3B). Collectively, these results indicate that TIGIT and PD-1 contribute to suppressed T-cell effector function. In line with this, intratumoral T cells cultured *in vitro* for 48 hours before activation of cytokine production had improved capacity to produce IL-2 in TIGIT^{+} CD8 T cells and TNF-α in PD-1^{+} CD4 T cells as compared to day 0 cultured cells (Supplementary Fig. S5). This suggested that the impaired effector function of TIGIT^{+} and PD-1^{+} T cells can be restored upon disruption of the *in vivo* immunosuppressive tumor microenvironment.

The TIGIT and PD-1 ligands are expressed in the tumor microenvironment

Co-inhibitory receptors need to be ligated to exert their suppressive functions. We next investigated the expression of the TIGIT and PD-1 cognate ligands, CD155/CD112 (18) and PD-L1/PD-L2 (24), respectively. Immunohistochemical analysis revealed expression of CD155 and CD112 on endothelial cells, including high endothelial venules and sinusoid endothelium, as well as on follicular dendritic cells (FDCs) in DLBCL, MCL and CLL (Fig. 4A; Supplementary Table S2). This is similar to what we observed in FL tumors (20). CD155 expression could also be observed in tumor cells from MCL and DLBCL (Fig. 4B). In contrast, PD-L1 demonstrated a different staining pattern, and was expressed by intratumoral macrophages as revealed by morphology and CD68 expression (Fig. 4A; Supplementary Table S2). Flow cytometry analysis confirmed CD155 and CD112 expression in tumor cells in 7 and 9 out of 18 DLBCL cases, and in 3 and 2 out of 10 MCL cases, respectively (Fig. 4C). In contrast, tumor cells were negative for CD112 and CD155 in all cases of FL, CLL and MZL (Fig. 4C). PD-L1/PD-L2 was only expressed by tumor cells in 2 of the DLBCL cases (Fig. 4C). These results suggest that TIGIT and PD-1 are likely to inhibit T-cell antitumor activity through interaction with the tumor microenvironment in FL, MCL, CLL, and MZL, as well as by ligand-expressing tumor cells in DLBCL.

Furthermore, as TIGIT competes for ligand binding with the co-stimulatory receptor CD226 (25), we also investigated expression of this receptor (Supplementary Fig. S6A). Our results revealed that CD8 T_{EM} cells, which displayed the highest expression of TIGIT (Supplementary Fig. S2), were CD226^{low} (<10% median expression across NHL; Supplementary Fig. S6B). In contrast, CD226 was frequently expressed in CD4 T cells, including FL T_{FH} cells as previously reported (Supplementary Fig. S6C) (20). Together, this

indicated that low expression of CD226 may play a role in TIGIT mediated inhibition of CD8 T cells in NHL.

Importantly, while PD-1 blocks signaling events downstream of the TCR by recruiting the protein tyrosine phosphatases SHP1 and SHP2 (26), TIGIT has been shown to inhibit effector function by recruiting the inositol 5-phosphatase SHIP1 (27). This, in context with our discovery that the majority of intratumoral CD8 and CD4 T_{EM} cells co-express TIGIT and PD-1, and the finding that $TIGIT^{+}PD\text{-}1^{+}$ T cells are poor producers of pro-inflammatory cytokines, indicates that dual blockade of these receptors might enable increased T-cell activity and tumor killing in NHL, potentially by recruitment of different phosphatases. Although not yet explored in lymphoma, a number of recent publications demonstrate synergistic activity of PD-1 and TIGIT co-blockade in preclinical cancer models. Interestingly, combined blockade of the two receptors resulted in complete responses in tumor mouse models of breast and colorectal cancers, while blocking only one receptor had little effect (15,16,28). Furthermore, a new anti-mouse TIGIT blocking antibody markedly increased survival of glioblastoma bearing mice when administered in combination with PD-1 blockade *in vivo*, and the cured mice developed long-term immunological memory protection upon re-challenge with tumor cells (28). To study the effect of TIGIT and PD-1 ligands *in vitro*, we introduced expression of PD-L1 and CD155 in the B-cell lymphoma cell line SU-DHL-4 (Supplementary Fig. S7A). However, FL T cells co-cultured with the original ligand-negative cell line had higher cytokine production as compared to T cells cultured in medium alone, and this difference was greater than between T cells co-cultured in the presence or absence of ligand-positive cells (Supplementary Fig. S7B). Importantly, a recent study demonstrated potent anti-tumor activity of PD-1 and TIGIT co-blockade in a mouse model, while TIGIT blockade had limited functional effect *in vitro* (28). Furthermore, TIGIT may influence several types of immune cells. In addition to inhibiting effector T cells (12), TIGIT has been shown to suppress immune responses by dampening NK cell activity (27) and by inducing immunoregulatory dendritic cells (11). Hence, blocking the TIGIT pathway in these cells may also be pivotal for efficient immunotherapy responses. Moreover, TIGIT is required for the B-cell helper function of circulating T_{FH} cells (29), suggesting that TIGIT might have different roles in distinct T-cell subsets. While T_{FH} cells are typically absent in DLBCL, CLL, MCL and MZL (Supplementary Fig. S4), $TIGIT^{+}$ T_{FH} cells are frequently found in FL (Supplementary Fig. S3-S4) (20). Thus, TIGIT blockade might also promote antitumor responses by reducing the tumor supporting effect of T_{FH} cells in FL. Furthermore, Tregs are

considered a suppressive barrier to potent antitumor responses in NHL, and we recently showed that Tregs from FL tumors express TIGIT (20). In contrast to the unresponsive phenotype of effector T cells, $TIGIT^+$ Tregs are highly functional cells and more potent suppressors of proinflammatory immune responses as compared to $TIGIT^-$ Tregs (13). Hence, immunotherapy using TIGIT blocking antibodies might promote immune responses in different ways, including restoring antitumor potential of effector T cells or reducing Treg immunosuppression.

Taken together, our results demonstrate frequent expression of TIGIT and PD-1in exhausted T cells from NHL tumors, and the presence of ligand positive cells in the tumor microenvironment (Supplementary Fig. S8). Overall, this study provides an overview of the co-inhibitory receptor landscape present in NHL and serves as a reference map of checkpoint receptors relevant to investigate in a clinical setting. Preclinical models demonstrate complete responses following co-blockade of TIGIT and PD-1 in solid cancer (15,16,28). Consistent with this, our findings support combinatorial blockade of TIGIT and PD-1, and further highlight the importance of characterizing the tumor microenvironment to fully understand mechanisms of immune escape.

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AUTHORSHIP CONTRIBUTIONS

S.E.J., K.H., R.L., A.K., E.B.S. and J.H.M. designed the study; S.E.J., K.B. and M.S.F. performed the research; S.E.J., K.B., K.H. and J.H.M analyzed results; K.B. revised patient diagnosis; Y.N.B., A.K., B.Ø., E.K. and H.H. provided patient samples; S.W., H.K. and B.B. performed genetic engineering; S.E.J. wrote the manuscript; J.H.M., E.B.S. and K.H. revised the manuscript, and all authors read and approved the final manuscript.

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FIGURE LEGENDS

Figure 1. TIGIT and PD-1 are frequently expressed by intratumoral T cells. Surface expression of 8 co-inhibitory receptors was analyzed in single cell suspensions from NHL tumors by flow cytometry. **(A)** Plots show $CD3^+$ T cells from one representative DLBCL sample. **(B)** Expression in $CD3^+$ T cells from FL (*n* = 19), DLBCL (*n* = 19), MCL (*n* = 11), CLL (*n* = 7), MZL (*n* = 2) and tonsils from healthy donors (*n* = 19). One data point represents a single donor. A number of the FL (*n* = 14) and tonsil (*n* = 10) specimens had been included in our previous study (20). Statistical differences calculated using Mann-Whitney non-parametric test and corrected for multiple testing; **p* < 0.05, ***p* < 0.01, ****p* < 0.001, *****p* < 0.0001.

Figure 2. TIGIT and PD-1 are co-expressed by intratumoral effector memory T cells. Surface expression of 8 co-inhibitory receptors was analyzed in single cell suspensions from NHL tumors by flow cytometry. T effector memory cells (T_{EM}) were identified as $CD45RA^-$ $CCR7^-$. **(A-B)** Surface expression of co-inhibitory receptors in CD8 **(A)** and in CD4 **(B)** T_{EM} in FL (*n* = 19), DLBCL (*n* = 19), MCL (*n* = 11), CLL (*n* = 7), MZL (*n* = 2) and tonsils from healthy donors (*n* = 19). One data point represents a single donor. **(C)** Identification of CD8 and CD4 T-cell subsets based on TIGIT and PD-1 expression. Plots show one representative DLBCL sample. **(D)** Distribution of CD8 and CD4 T_{EM} with differential expression of TIGIT and PD-1 in FL (*n* = 5), DLBCL (*n* = 19), MCL (*n* = 11), CLL (*n* = 7), MZL (*n* = 2) and tonsils (*n* = 7). T-cell populations identified by gating strategy shown in (C). Statistical differences between tonsils and NHL calculated using Mann-Whitney non-parametric test and corrected for multiple testing; **p* < 0.05, ***p* < 0.01, ****p* < 0.001, *****p* < 0.0001.

Figure 3. PD-1 and TIGIT expression correlate with low cytokine production. Intracellular TNF-α, IFN-γ and IL-2 was analyzed by flow cytometry. **(A)** Cytokine production in $CD3^+$ T cells correlated with TIGIT and PD-1 expression. Plots show one representative DLBCL sample. **(B)** Cytokine production in CD4 and CD8 T cells from FL (*n* = 4), DLBCL (*n* = 4), MCL (*n* = 3) and CLL (*n* = 4). The T-cell populations were identified by the same gating strategy as shown in Fig. 2C. Each data point represents a single donor. Background values as determined in unstimulated controls were subtracted from the stimulated samples. Statistical differences calculated using non-parametric Friedman's test and corrected for multiple testing; **p* < 0.05, ***p* < 0.01, ****p* < 0.001, *****p* < 0.0001.

Figure 4. The TIGIT and PD-1 ligands are expressed in NHL. Ligand expression was characterized by immunohistochemistry and flow cytometry. **(A-B)** Immunohistochemical staining of DLBCL, MCL and CLL frozen tissue sections using antibodies against CD155, CD112, PD-L1 and CD68. The tissue sections are closely neighbored to each other, enabling the comparison of identical structures. **(A)** CD155 and CD112 are expressed by endothelial cells while PD-L1 is found on macrophages, as confirmed by staining with macrophage marker CD68. Image objective x20. **(B)** MCL and DLBCL cases demonstrating CD155 expression in lymphoma cells (arrows in left and right panel), in addition to endothelial cells (arrow head left panel) and intrasinusal histiocytes/macrophages (arrow heads right panel). **(C)** Flow cytometry analysis of CD155, CD112 and PD-L1/PD-L2 surface expression in tumor cells from FL (n = 10), DLBCL (n = 18), MCL (n = 10), CLL (n = 6) and MZL (n = 2), and non-malignant B cells from tonsils (n = 8) analyzed by flow cytometry.

Figure 1

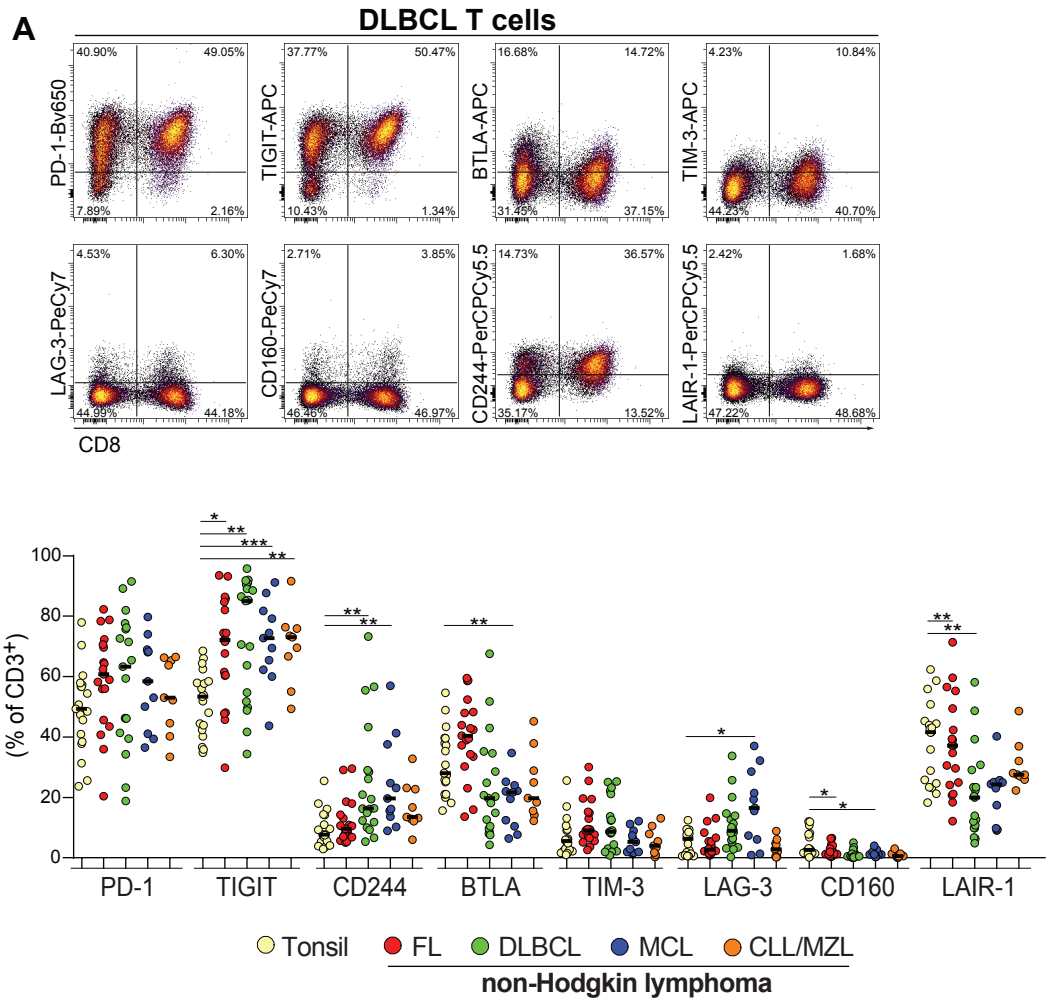

A
DLBCL T cells
PD-1-Bv650
TIGIT-APC
BTLA-APC
TIM-3-APC
LAG-3-PeCy7
CD160-PeCy7
CD244-PerCPCy5.5
LAIR-1-PerCPCy5.5
CD8
B
Receptor-expressing cells (% of CD3+)
PD-1
TIGIT
CD244
BTLA
TIM-3
LAG-3
CD160
LAIR-1
Tonsil
FL
DLBCL
MCL
CLL/MZL
non-Hodgkin lymphoma

Figure 2

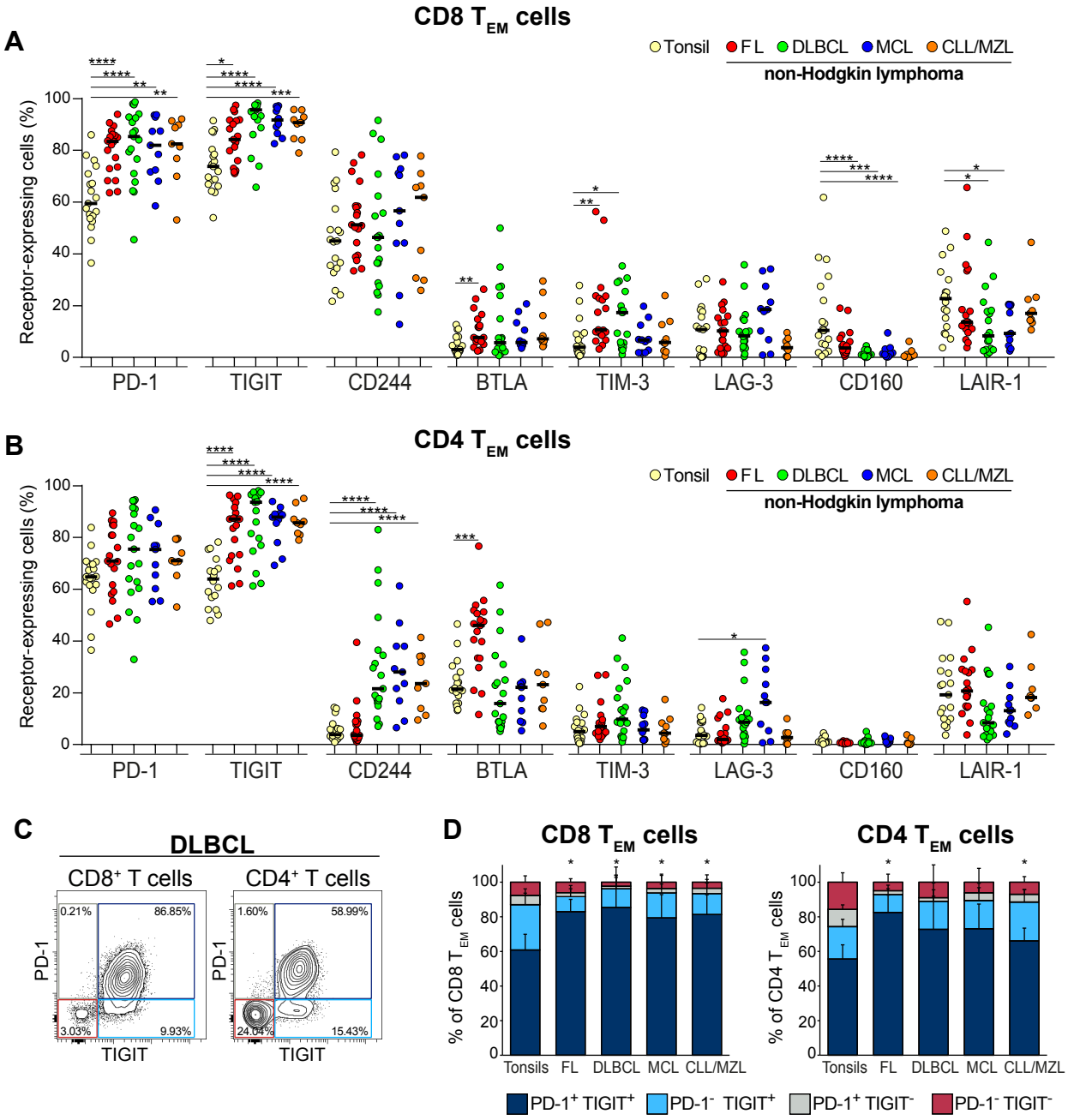

A
CD8 T EM cells
Tonsil
FL
DLBCL
MCL
CLL/MZL
non-Hodgkin lymphoma
Receptor-expressing cells (%)
PD-1
TIGIT
CD244
BTLA
TIM-3
LAG-3
CD160
LAIR-1
B
CD4 T EM cells
C
DLBCL
CD8+ T cells
CD4+ T cells
0.21%
86.85%
3.03%
9.93%
1.60%
58.99%
24.04%
15.43%
D
CD8 T EM cells
CD4 T EM cells
% of CD8 T EM cells
% of CD4 T EM cells
Tonsils
FL
DLBCL
MCL
CLL/MZL
PD-1+ TIGIT+
PD-1- TIGIT+
PD-1+ TIGIT-
PD-1- TIGIT-

Figure 3

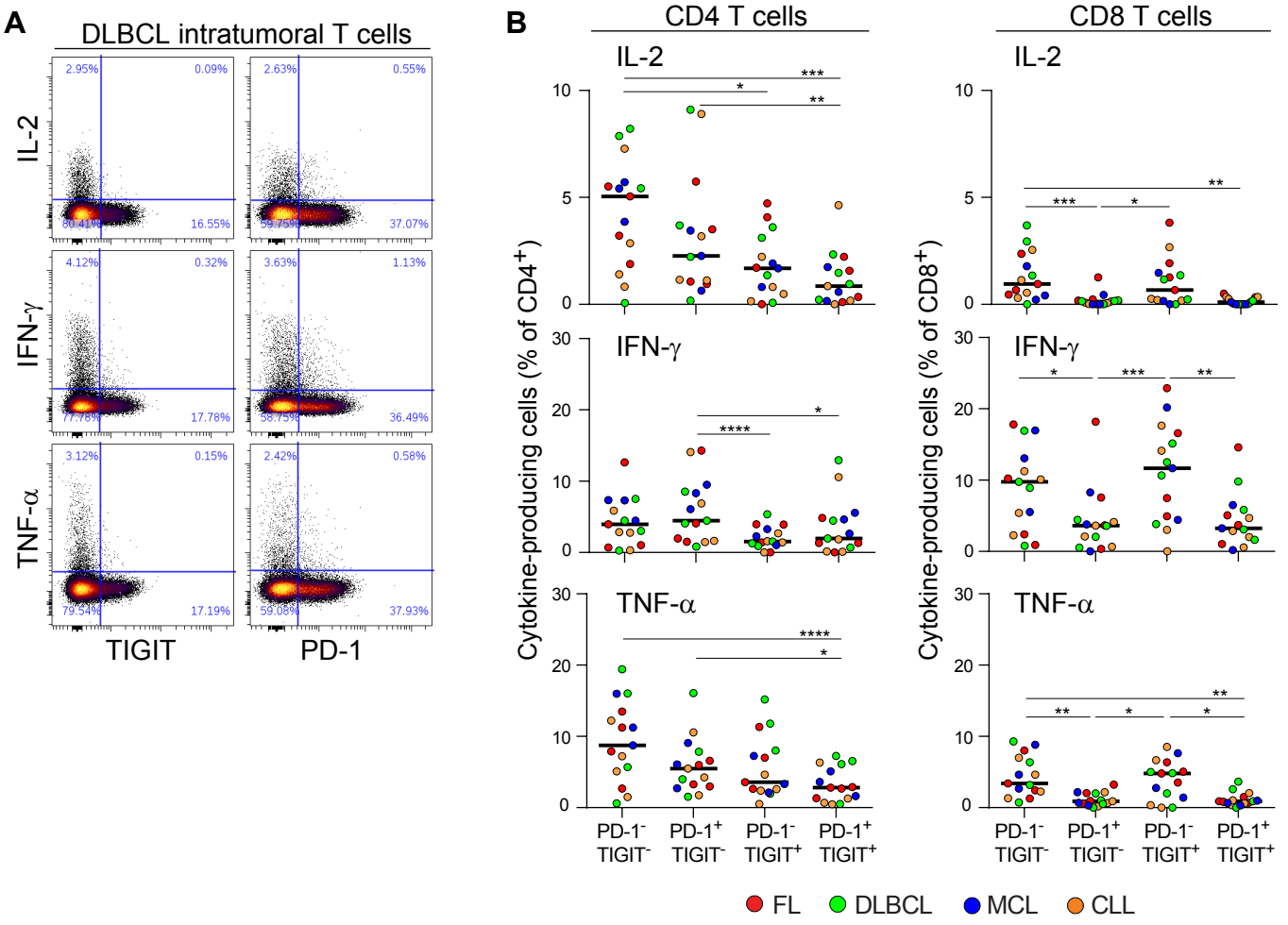
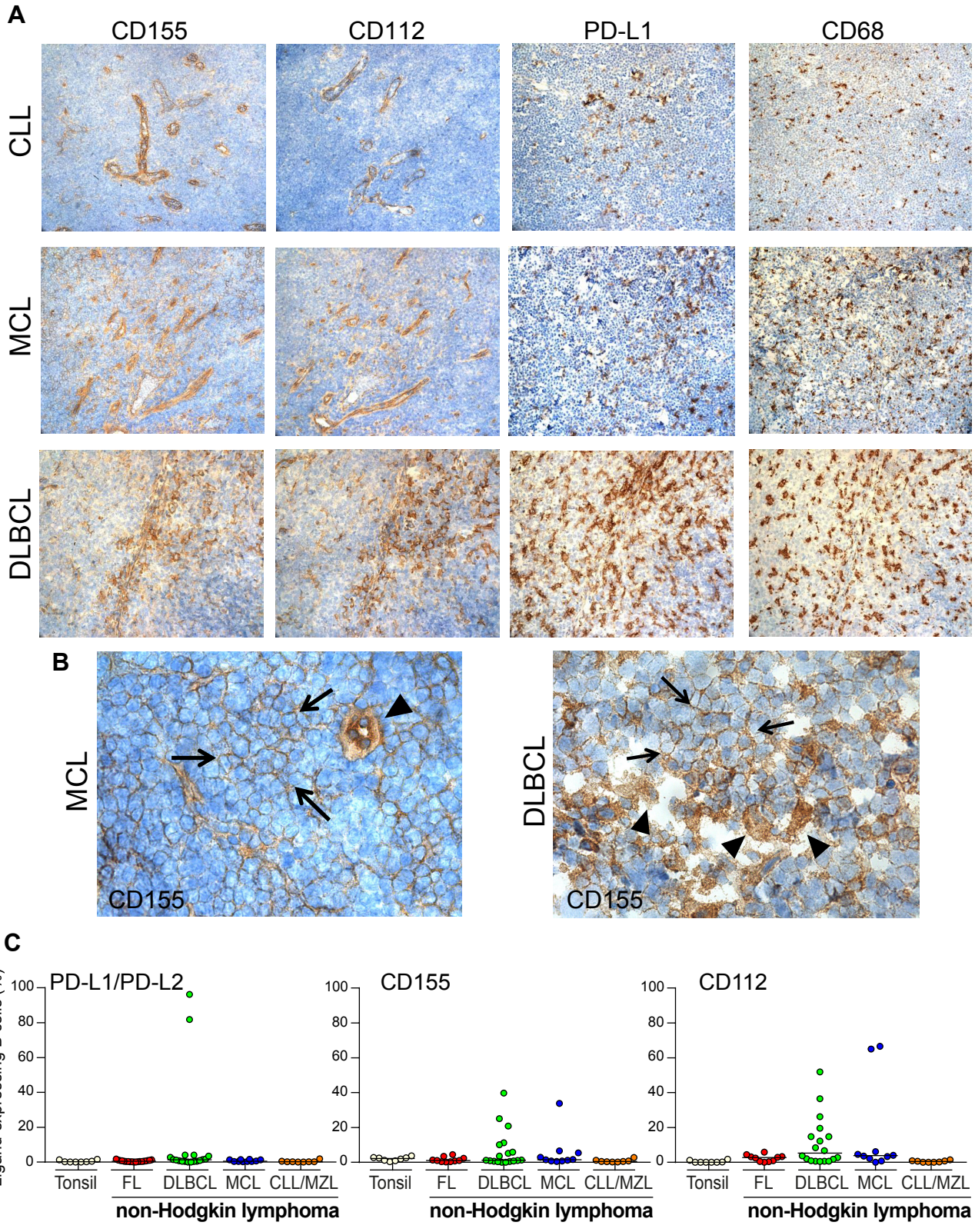

A
DLBCL intratumoral T cells
IL-2
IFN-γ
TNF-α
TIGIT
PD-1
2.95%
0.09%
16.55%
2.63%
0.55%
37.07%
4.12%
0.32%
17.78%
3.63%
1.13%
36.49%
3.12%
0.15%
17.19%
2.42%
0.58%
37.93%
B
CD4 T cells
CD8 T cells
IL-2
IFN-γ
TNF-α
Cytokine-producing cells (% of CD4+)
Cytokine-producing cells (% of CD8+)
PD-1- TIGIT-
PD-1+ TIGIT-
PD-1- TIGIT+
PD-1+ TIGIT+
FL
DLBCL
MCL
CLL

Figure 4


A
CD155
CD112
PD-L1
CD68
CLL
MCL
DLBCL
B
MCL
CD155
DLBCL
CD155
C
Ligand-expressing B cells (%)
PD-L1/PD-L2
CD155
CD112
100
80
60
40
20
0
Tonsil
FL
DLBCL
MCL
CLL/MZL
non-Hodgkin lymphoma