Role of the microenvironment across histological subtypes of NHL

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LEARNING OBJECTIVES

1. To understand how the dynamic interplay between lymphoma B cells and their tumor microenvironment triggers the building of a supportive niche integrating immune escape mechanisms and B-cell survival and proliferation signals.

2. To recognize the main limitations, challenges, and open questions in the field of tumor lymphoma microenvironment

ABSTRACT

While the recent progresses of next-generation sequencing strategies have revealed the genetic landscape of B-cell non-Hodgkin lymphoma, tumor microenvironment has been increasingly recognized as crucial to sustain malignant B-cell survival, growth, subclonal evolution, and drug resistance. Tumor niche is made up of a dynamic and organized network of strongly heterogeneous immune and stromal cell subsets, characterized by specific phenotypic, transcriptomic, and functional features. Non-malignant cell recruitment and plasticity are dictated by lymphoma B cells, which convert their surrounding microenvironment into a supportive niche. In addition, they are also influenced by the crosstalk between the various components of this niche. In agreement, the B lymphoma subtype is a key determinant of the tumor niche organization, but genetic alteration pattern, tumor localization, stage of the disease, treatment strategy may also modulate its composition and activity. Moreover, the complex set of bidirectional interactions between B cells and their microenvironment has been proposed as a promising therapeutic target with the aim to reinforce anti-tumor immunity and/or to abolish the lymphoma promoting signals delivered by the tumor niche.

INTRODUCTION

B-cell non-Hodgkin's Lymphomas (B-NHL) are a group of highly heterogeneous tumors characterized by a disseminated infiltration of lymphoid structures by malignant mature B cells. Each lymphoma subtype could be assigned to a peculiar stage of B-cell differentiation and harbors a panel of genetic alterations sustaining specific transformation pathways and disease evolution[1]. Follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL) account together for about 70% of B-NHL and are derived from germinal center (GC) B cells at various stages of the GC transit, namely centrocytes of the GC-light zone for FL and GC B cell (GCB)-like DLBCL, and committed post-GC plasmablasts for DLBCL of the activated B cell (ABC)-phenotype. Histological transformation of indolent FL to aggressive lymphoma, more closely related to GCB-DLBCL, occurs in about 35% of cases and is associated with poor outcome. Genome-wide profiling has recently shed new lights on the mutational landscape of both FL and DLBCL thus providing considerable advancement in our understanding of lymphomagenesis. However tumors are now widely recognized as complex and dynamic ecosystems supporting coevolution of malignant cells and their surrounding microenvironment, whose quantitative and qualitative composition influence tumor initiation, growth, progression, immune escape, and drug resistance. Interestingly, FL and DLBCL are characterized by different patterns of tumor niche organization, a phenomenon that could contribute to their different clinical course and should be considered for the development of new therapeutic strategies[2]. In agreement, it is virtually impossible to maintain FL B cells *in vitro* whereas numerous DLBCL cell lines of both GC and ABC phenotypes have been successfully established. This review will focus on these two frequent B-NHL

subsets in order to highlight the main recent advances and unsolved questions on the role of microenvironment in lymphomagenesis.

LYMPHOMA MICROENVIRONMENT CHALLENGES

FL is characterized by a long preclinical stage, an indolent clinical course with multiple relapses, and retains a substantial degree of dependence on a specific GC-like microenvironment including in particular specialized subsets of $CD4^{pos}$ T cells, stromal cells, and macrophages[3]. Moreover, this lymphoid-like microenvironment is ectopically induced in FL-invaded bone marrow (BM) where paratrabecular nodular aggregates of malignant B cells are enriched for functional lymphoid-like stromal cells and $CD4^{pos}$ T cells[4]. Accordingly, immunohistochemical and transcriptomic studies have provided a large panel of predictive biomarkers reflecting the quantitative and qualitative composition as well as the spatial organization of FL lymph node (LN) infiltrating immune cells[3]. FL B-cell cytological grade, proliferation rate, and subclonal evolution differ between LN and BM suggesting that trafficking within different microenvironments could impact FL phenotypic and molecular heterogeneity. DLBCL is described as less dependent on its microenvironment in agreement with a complete disorganization of normal lymphoid structure. Interestingly, $G\alpha 13$-dependent signaling is crucial to maintain normal GC B-cell confinement and this pathway is frequently mutated in GC-DLBCL and transformed FL, allowing malignant B-cell dissemination and favoring microenvironment-independent B-cell survival[5,6]. However, besides the widely used GC/ABC classification reflecting malignant B-cell features, two gene-expression profiling studies have highlighted another level of DLBCL biological heterogeneity underlying the role of the microenvironment. In the first one, a host response signature was identified, related to immune activation, and

was associated to unique clinical features[7]. In the second one, a prognostically favorable stromal-1 signature, associated with extracellular-matrix deposition and myeloid cell infiltration, and a prognostically unfavorable stromal-2 signature, reflecting tumor blood-vessel density, were characterized[8]. These studies suggest that microenvironment features contribute to FL/DLBCL pathogenesis. However, they have shown highly contradictory results concerning their impact on patient outcome, at least partly due to the heterogeneity in the patient cohorts and treatment schedules but also to substantial technical hurdles limiting data reproducibility. In addition, such descriptive studies do not provide any mechanistic insights into the functional role of lymphoma cell niches.

The main biological limitation to a comprehensive analysis of microenvironment in B-cell malignancies is related to the heterogeneity and plasticity of the numerous and sometimes very rare cell subsets involved. Such diversity could not be efficiently capture by the low resolution tools classically used, a phenomenon recently amplified by the reduced size of available biological samples related to the increasing use of fine-needle biopsies. As an example, multicolor flow cytometry/cell sorting strategies and *in vitro* assays were required to pinpoint that the majority of PD-1-expressing $CD4^{pos}$ T cells in FL are fully functional PD-1^{hi} $CXCR5^{hi}$Tim-3^{neg} follicular helper T cells (Tfh) [9,10], opposing the previous hypothesis that they represent exhausted T cells[11]. Based on such more comprehensive technical approaches, the main open questions challlenge i) the quantitative, qualitative, spatial, and functional heterogeneity of lymphoma microenvironment, taking into account tumor genetic alterations, tumor localization (LN *versus* BM), stage of the disease, and impact of treatment; ii) the origin of lymphoma microenvironment subsets, including the identification of precursor cells and commitment pathways; iii) the clinical relevance

of the prognostic/stratification markers relying on lymphoma microenvironment characterization and its potential as a target for new therapeutic options. These major fields of investigation should be considered for both the anti-tumoral and pro-tumoral facets of tumor microenvironment.

ANTI-TUMORAL MICROENVIRONMENT

FL has long been considered as immune responsive based on high response rates to anti-CD20 monoclonal antibodies (mAbs) associated with a long-lasting vaccinal effect, good biological responses to idiotype vaccines, and interesting response rates in recent clinical trials involving immunomodulatoy drugs such as lenalidomide or immune checkpoint inhibitors. In DLBCL, immunochemotherapy is also the standard of care and soluble PD-L1 (sPD-L1) has recently emerged as a powerful prognostic biomarker[12] suggesting a role for the immune system in the control of disease progression. Several immune cell subsets contribute to this anti-tumor activity and conversely lymphoma cells escape or dampen antitumor immunity through modifications of malignant B-cell phenotype and induction or recruitment of a suppressive microenvironment (Figure 1).

Immune response

Cytotoxic lymphoid cells, including $CD8^{pos}$ T cells, $\gamma\delta$T cells, and NK cells, play a central role in anti-lymphoma immunity. Importantly, the cells of origin of B-cell lymphomas are professional antigen-presenting cells and constitutively express MHC class II molecules involved in antigen presentation to $CD4^{pos}$ T cells, a crucial step for the initiation of immune response. In parallel, dendritic cell (DC) frequency is reduced in FL, as previously described in several tumor models[13]. As expected, a high $CD4^{pos}$

T-cell infiltration, essentially of memory phenotype, has been correlated with increased overall survival and lower malignant B-cell proliferation rate in DLBCL patients[14]. In FL, where different populations of $CD4^{pos}$ T cells could restrain or favor B-cell growth, increased in $CD3^{pos}$ and $CD8^{pos}$ but not $CD4^{pos}$ T-cell infiltrates have been reproducibly correlated to a better clinical outcome[15]. Moreover, a rich infiltrate of $CD8^{pos}$ T cells expressing the pore-forming protein granzyme B and engaged in lytic-like structures with FL B cells was detected at the FL follicle border[16]. Even if few studies have directly addressed the question of lymphoma-infiltrating NK cells *in situ*, they contribute to the activity of anti-tumor antibodies through antibody-dependent cell cytotoxicity (ADCC), as highlighted by the impact of the CD16/FcRγIIIA-V158F polymorphism on the clinical response to Rituximab. Finally Vγ9δ2 T cells recognizing tumor phosphoantigens are able to kill lymphoma B cells *in vitro*[17]. Interestingly, whereas they represent a minority of γδ T cells in peripheral blood from healthy individuals, Vδ1 T lymphocytes, responding to stress-associated ligands, are expanded in FL and DLBCL patients[18,19]. Of note, $V\delta2^{neg}$ T cells are known to express CD16 in CMV-infected individuals and to display ADCC potential, suggesting that they could contribute to anti-CD20 activity in B-cell NHL[20].

Tumor-associated macrophages (TAMs) exhibit a dual role in FL and DLBCL pathogenesis, as underlined by the opposite predictive value of TAM content depending on treatment schedule[3,21]. Whereas a high number of TAMs was essentially associated with poor outcome in patients treated by chemotherapy, elevated CD68 and/or CD163 staining was associated with a favorable prognosis when patients were treated with Rituximab. These data could be related to the demonstration that $CD163^{pos}$ M2 polarized macrophages display high phagocytic capacity toward rituximab-opsonized targets[22]. Interestingly, the blockade of the

"don't eat me" molecule CD47, overexpressed on FL and DLBCL B cells, increases *in vitro* and *in vivo* the phagocytic activity of macrophages expressing the inhibitory receptor signal regulatory protein (SIRP)-α suggesting that tumor cells exploit the suppressive CD47-SIRPα axis to evade macrophage-mediated destruction[23].

Overall, activated cytotoxic and phagocytic cells from innate and adaptive immunity directly kill malignant B cells and release inflammatory cytokines that could contribute to their reciprocal activation. However, this antitumor immune response is counteracted by tumor escape mechanisms.

Mechanisms of immune evasion and subversion

The first mechanism of immune evasion is the reduction of tumor immunogenicity related to a panel of genetic alterations triggering a lack of malignant B cell recognition by $CD4^{pos}$ T, $CD8^{pos}$ T, and NK cells[2,24] (Table 1).

The second driving mechanism of tumor escape is the reduced T cell anti-tumoral activity. FL-infiltrating T cells display a decreased F-actin polymerization at the immunological synapse[25] and an impaired LFA-1-dependent motility[26], indicating alteration of cytoskeleton-dependent T-cell activation. These defects, also detected in DLBCL-infiltrating T cells, could be induced *in vitro* in healthy donor T cells by direct contact with malignant B cells and could be reversed, at least partly, by lenalidomide. It was proposed that expression of multiple cell-surface inhibitory molecules by lymphoma cells, including CD200, PD-L1, or HVEM contribute to the impairment of T-cell actin dynamic across hematologic B-cell malignancies[27]. However, it should be keep in mind that PD-L1 expression is restricted to a subset of 20-30% DLBCL, essentially of the non-GCB subtype, in relationship with different genetic alterations and an inferior overall survival[28]. Conversely, whereas infiltrating myeloid cells could

express PD-L1 in both FL and DLBCL, FL B cells are PD-L1^{neg} and display inactivating genetic alterations of HVEM in 30-40% of cases[29]. Regardless of inhibitory receptor ligand expression by malignant cells and/or by their microenvironment, exhausted T cells harboring the corresponding receptors infiltrate B-cell lymphomas. TIM-3 and LAG-3 have emerged as good markers for functionally impaired FL-infiltrating T cells whereas PD-1 expression is not sufficient to distinguish exhausted CD4^{pos} T cells from immunologically competent Tfh[9,30,31]. Finally, CD70 upregulation is associated with FL memory T-cell exhaustion and predicts inferior patient outcome[32]. FL B-cell-derived IL-12 was proposed as a driving mechanism of TIM-3 and LAG-3 induction[30,33], while TGF-β triggers exhaustion of effector memory T cells[32]. Interestingly, NK and Tγδ cells also express some inhibitory receptors, including PD-L1 and BTLA, and could thus be subverted by malignant B cells or TAM expressing the corresponding ligands. Moreover, lectin-like transcript 1 (LLT1) was recently identified as a marker of normal and malignant GC B cells, including FL and GC-DLBCL, and was shown to dampen NK cell functions through interaction with its receptor, CD161[34].

Besides the loss of T/NK cell activation, FL/DLBCL biopsies are also characterized by an expansion of immune suppressive cells in particular Treg. Malignant B cells have been shown to produce high amounts of the Treg recruiting chemokine CCL22 in response to Tfh-derived IL4 and CD40L signals[35] and to skew the balance of Th polarization towards FOXP-3^{pos} Treg and away from Th17[36]. Expression of CD70, CD80/86, or membrane TGF-β has been involved in this process. Lymphoma-infiltrating Treg efficiently inhibit both CD4^{pos} and CD8^{pos} T-cell proliferation and Treg frequency is inversely correlated to CD8^{pos} T-cell frequency in B-cell NHL[37]. A specificity of regulatory T-cell compartment in FL unlike DLBCL is that, as described

for effector Th cells, it includes both CXCR5^{neg}PD-1^{neg} classical Treg and CXCR5^{pos}PD-1^{pos} follicular regulatory T cells (Tfr)[10]. Tfr localize within normal GC and inhibit Tfh-mediated B-cell activation and antibody production thus controlling the dynamic and extent of GC reaction[38]. Fully functional Tfr are strongly expanded within FL cell niche compared to reactive LN and ICOSL^{pos} FL B cells have been involved in this Tfr enrichment[39]. Their role in FL pathogenesis is currently unknown and the prognostic value of FOXP-3^{pos} Treg count in FL remains controversial[3].

Besides Treg, myeloid cells could also contribute to immune suppression. In all B-cell lymphoma subtypes, TAMs have been shown to release interleukin-4-induced gene 1 (IL4I1) and indoleamine-2,3 dioxygenase (IDO) whereas IL4I1 is also produced by malignant B cells in FL and primary mediastinal large B-cell lymphomas (PMBL) and IDO by DLBCL B cells[40,41]. These two amino acid-degrading immunosuppressive enzymes are involved in the expansion of Treg and the blockade of effector T-cell proliferation and cytotoxicity. More recently, immature granulocyte and monocyte subsets have demonstrated their immunosuppressive function in cancers and inflammatory diseases and are recognized as myeloid-derived suppressor cells (MDSCs). Circulating CD14^{pos}HLA-DR^{lo} monocytic-MDSC count is elevated in DLBCL patients, and correlates to clinical outcome and Treg number[42]. IL-10 has been proposed as an inducer of M-MDSC expansion in B-NHL[43] while MDSC-dependent T-cell suppression was attributed to IL-10, S100A12, and PD-L1 expression[42]. Of note, even if their number was not shown to predict lymphoma patient prognosis, granulocytic-MDSCs are also expanded in DLBCL patients, and correlate with the plasma level of the immunosuppressive enzyme arginase 1. The biological activity of MDSCs inside tumors and their relationship with TAMs remain to be explored. Finally, an interesting study has proposed that lymphoma-infiltrating

endothelial cells overexpress TIM-3 thus facilitating immune evasion[44] and FL-infiltrating stromal cells have been shown to produce more PGE2, a well-known immunosuppressive factor, than their normal counterpart[45].

In conclusion, B-NHL immune microenvironment could be considered as a network of exhausted/suppressed anti-tumor cell subsets and recruited/activated suppressive cell compartments that dynamically interact with each other. Malignant B cells contribute to the organization of this favorable niche, as shown by the selection of lymphoma cells harboring genetic and phenotypic features favoring tumor escape.

PRO-TUMORAL MICROENVIRONMENT

Besides the immune activation/immune escape active loop that supports continuous lymphoma immunoediting, B-NHL microenvironment is also organize to provide survival and growth signals to malignant B cells through specialized immune and stromal cell subsets exhibiting specific polarization and activation profiles (Figure 2).

Direct lymphoma-promoting signals

Cancer-associated fibroblasts (CAFs) have been recognized as playing critical roles in tumor development, and progression in various solid tumor models. CAFs organize a heterogeneous network of activated reprogrammed myofibroblasts exhibiting specific phenotype, proliferation rate, migration propensity, gene expression profile, and epigenetic features. Owing to the demonstration that the survival and drug resistance of FL B cells could be increased by co-culture with stromal cells, FL CAFs have focused more attention than their DLBCL counterpart. FL CAFs display phenotypic features of lymphoid stromal cells within invaded LN and BM[46]. In agreement, stromal cells obtained from FL BM exhibit a specific gene expression

profile including enrichment for a lymphoid stroma signature associated with an increased capacity to sustain FL B-cell growth[47]. BM and LN stromal cells have been shown to prevent spontaneous and drug-induced apoptosis of NHL B cells *in vitro* through several mechanisms[4,48,49] including the production of hedgehog (Hh) ligands; the induction of miR-181a and decrease of miR-548m in malignant B cells leading to a downregulation of the proapoptotic protein BIM and an activation of a pro-survival c-MYC/HDAC6 loop; the activation of NF-κB pathway through poorly characterized stimuli including the release of BAFF; and the upregulation of ABC transporters triggering multidrug resistance. Of note, even if DLBCL malignant B cells produce autocrine Hh ligands, thus contributing to their decreased stroma-dependence, co-culture of DLBCL with stromal cells further reinforce drug tolerance and NF-κB activation independently of NF-κB genetic alterations and at least in part through the release of Hh ligands and BAFF[50]. Lymphoid stromal cell-derived chemokines, including CXCL13, CCL19/CCL21, and CXCL12, contribute to lymphoma B cell homing and retention and CXCL12 was recently shown to be specifically upregulated within LN and BM FL stromal cell niches[51]. Of note, a majority of functional studies have been performed using stromal cells maintained in long-term *in vitro* cultures and a detailed *in situ/ex-vivo* characterization of FL and DLBCL CAFs is still lacking.

Myeloid cells are also a major component of FL/DLBCL cell niches. FL-TAM overexpress and transpresent IL-15 and collaborate with CD40L-expressing FL-Tfh to trigger STAT5 activation and FL B-cell growth[52]. In DLBCL, BAFF was proposed as another monocyte-derived survival factor[53]. In addition, DLBCL B cells were shown to produce IL-8 and to recruit APRIL-expressing neutrophils able in turn to protect them from spontaneous cell death *in vitro*[54]. Conversely, direct contact with neutrophils was not efficient to rescue primary FL B cells from apoptosis[55].

Importantly, FL-TAM were recently proposed to be involved in FL BCR activation. Specifically, although less than 25% of FL BCR are supposed to be self-reactive, FL BCR is characterized in most, if not all, FL cases by the introduction and positive selection of N-glycosylation sites in the variable region of immunoglobulin genes[56]. The acquisition of these specific sequence motifs, that are very rare in normal B cells, is an early genetic event in FL pathogenesis but it does not provide any B-cell intrinsic advantage to malignant cells. Surprisingly, the glycan chains added to these sites, conversely to glycans of the Fc region of the same molecules, unusually terminate in a high mannose, allowing the interaction with DC-SIGN^{pos} FL-TAM that trigger universal long-lasting BCR aggregation and activation[57]. This process supports FL B-cell survival *in vitro* and could be abrogated by pharmacologic BCR inhibitors. In DLBCL, no role for cells of the microenvironment has been proposed in BCR crosslinking. In ABC-DLBCL, some recurrent genetic alterations, such as mutations in CARD11, provide autonomy from external BCR signal, while others, in particular those affecting CD79A/CD79B, increase the amplitude of the external BCR activation, potentially initiated by self-antigen recognition, thus contributing to an antigen-dependent chronic BCR signaling[58]. Conversely, GC-DLBCL utilize an antigen-independent tonic BCR signaling.

Finally FL microenvironment is heavily enriched for CD4^{pos} T cells displaying phenotypic and transcriptomic features of Tfh cells, the specialized T-cell subset involved in high-affinity GC B-cell selection, amplification, and differentiation[10]. Importantly, Tfh are virtually absent from DLBCL cell niche. FL-Tfh are characterized by unique gene expression and cytokine secretion profiles, partly explained by the amplification of a CD10-expressing Tfh subset able to directly sustain FL B-cell survival, in particular through IL-4 and CD40L signals[59]. As discussed above, CD40L

signal also confers IL-15 sensitivity to B cells through induction of STAT5 expression and activation and cooperates thus with FL-TAM to support FL cell growth[52].

Collectively, these studies demonstrate that B-NHL supportive microenvironment is made up of a specific combination of stromal cell, myeloid cell, and $CD4^{pos}$ T cell subsets.

Mechanisms of tumor-supporting cell polarization and activation

The mechanisms underlying supportive microenvironment recruitment, amplification, and commitment are an intense matter of interest since they could provide relevant new therapeutic targets. Malignant B cells themselves can endow their surrounding niche with supportive properties through a dynamic reciprocal activation program. In particular, loss-of-function alterations of TNFRSF14/HVEM in FL have been shown to trigger both B-cell autonomous activation, in cooperation with BCR signaling, and B-cell extrinsic activation of lymphoma microenvironment[29]. In a FL mouse model based on the simultaneous deregulation of BCL2 and inactivation of HVEM, Tfh, that express very high level of the HVEM inhibitory receptor BTLA, were amplified and produced higher amount of IL-4, TNF, and lymphotoxin (LT). Interestingly, FL patients displaying HVEM inactivation exhibit increased Tfh infiltration and IL-4-dependent STAT6 phosphorylation *in situ*, thus validating the impact of HVEM genetic alteration on the composition of tumor microenvironment. TNF and LT, the two non-redundant factors involved in lymphoid stroma differentiation and maintenance, were also upregulated in malignant B cells in HVEM-deficient mice and lymphoid stromal cells, known to trigger FL B-cell survival, were over-activated. In agreement, primary human FL B cells contribute in a TNF-dependent manner to the differentiation of lymphoid stromal cells and to their higher secretion of CCL2 and IL-

8[47,55]. However, neither co-culture with malignant B cells nor treatment with TNF is able to upregulate CXCL12 in human stromal cells[51], asking the question of the other mechanisms involved in FL stroma polarization.

As discussed previously, FL-Tfh are characterized by an upregulation of IL-4, TNF, and LT compared to Tfh obtained from reactive tonsils[10]. Interestingly, these 3 molecules affect stromal cell phenotype. IL-4, as well as FL-Tfh themselves, was recently highlighted as CXCL12 inducer in stromal precursors and lymphoid stromal cells[51]. Of note, stromal cells were also proposed to support the viability of FL-Tfh even if the role of lymphoid stroma in Tfh activation and functional heterogeneity remains to be explored[60]. Tfh-derived IL-4 also contributes indirectly to FL pathogenesis through the upregulation of DC-SIGN on macrophages and IgM on FL B cells, thus favoring the TAM/malignant cell crosstalk[57].

Finally, besides their direct B-cell supportive effects, stromal cells also contribute to the polarization and organization of FL microenvironment. In particular FL stromal cells can recruit and interact with innate immune cells, including monocytes and neutrophils, through an overexpression of CCL2 and IL-8[47,55]. In agreement, these two chemokines are overexpressed in FL invaded BM compared to normal BM. Interestingly, FL-MSC protect recruited neutrophils from apoptosis and convert recruited monocytes into proangiogenic and anti-inflammatory TAM-like cells[47,55]. Both TAN and TAM cooperate then with stromal cells to trigger FL B cell survival.

CONCLUSIONS AND PERSPECTIVES

With the introduction of next-generation sequencing, the genetic landscape of B-cell NHL has rapidly been unraveled in recent years, thus placing a spotlight on the role of these genetic alterations in lymphomagenesis. However, microenvironment

emerges now as a key determinant of lymphoma development and evolution. In addition, progressive identification of pro- and anti-tumoral signals delivered by the dynamic cell network surrounding malignant B cells have paved the way for new therapeutic approaches, aiming at improving the function of immune effector cells and/or at disrupting the crosstalk between tumor cells and their supportive niche. The general lack of description of the heterogeneity of microenvironment subsets will be solved rapidly by the development of single-cell strategies, including CyTOF and RNAseq approaches but also exhaustive TCR repertoire. However, new questions emerge. First, the roles of extracellular matrix components, mechanical properties, and niche microarchitecture have not been fully appreciated. Second, after deciphering differences between microenvironment composition depending on lymphoma subtypes, lymphoma patients, and lymphoma localizations, the dissection of intra-tumor heterogeneity also emerges as an important facet of the understanding of B-NHL niche, looking for spatial segregation of immune and stromal cells into discrete functional zones sustaining B-NHL proliferation or cancer progenitor cells maintenance. Finally the mechanisms of B-cell/microenvironment crosstalk remain elusive, and the role of extracellular vesicle exchanges, the impact of genetic alterations on niche composition and conversely the role of the pro- and anti-tumoral microenvironment on the selection of malignant B-cell subclones have just started to be studied. The biggest roadblock to solve these issues is the inadequacy of testing models. The development of new tools, including histo-cytometry on whole tissues, 3D tumor organoids mixing various cell subsets, or relevant lymphoma mouse models mimicking genetic events and microenvironment organization, will be mandatory to better understand B-NHL pathogenesis and to test new drug efficacy and mechanisms of action.

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Table 1: Mechanisms of immune escape in FL and DLBCL

Immune defect	Proposed mechanisms
Immune evasion	
Lack of recognition by $CD4^{pos}$ T cells	Loss of MHC class II • *MHC class II deletion* • *CREBBP mutations* • *Plasmablastic differentiation* • *Mutational landscape evolution*
Lack of recognition by $CD8^{pos}$ T cells	Loss of MHC class I • *β2M mutation or deletion* • *Mutational landscape evolution*
Lack of recognition by NK cells	Loss of CD58 • *Mutation or deletion*
Decreased phagocytosis	Overexpression of CD47
Immune subversion	
Impaired T/NK activity	Expression of inhibitory molecules (CD200, PD-L1, HVEM, LLT1) Production of IL-12 and TGFβ Production of IDO and IL4I1
Treg/Tfr amplification	Production of CCL22 Expression of CD70, CD80/CD86, ICOSL, TGFβ
Amplification of myeloid suppressive cells	Production of IL-10
Amplification of suppressive non-hematopoietic cells (endothelial, stromal)	Unknown

LEGENDS TO FIGURES

Figure 1: Immune escape mecanisms in B-NHL.

Malignant B cells progressively loss surface molecules involved in recognition by CD4 (MHC II), CD8 (MHC I), and NK (CD58) cells whereas they variably overexpress inhibitory receptors including PD-L1, LLT1, HVEM, and CD47, the ligands for PD-1, CD161, BTLA, and SIRP-α, and produce the inhibitory enzymes IDO and IL-4I1. The combination of these mechanisms allows them to avoid killing by cytotoxic cells and phagocytosis by tumor-associated macrophages (TAM). In addition, they contribute to Treg recruitment and differentiation, and to exhaustion of T effector cells through the release of CCL22, TGF-β, and IL-12 and the expression of ICOS-L and CD80/CD86. MDSC, myeloid-derived suppressor cell; NHL, non-Hodgkin lymphoma; pAg, phosphoantigen.

Figure 2: Tumor-supporting niche in FL

Three main cell subsets have been shown to support FL B-cell growth: i) FL tumor-associated macrophages (TAM) overexpress IL-15 that triggers STAT5-dependent FL B cell activation, and DC-SIGN that aggregates FL mannosylated BCR; ii) expanded FL-Tfh activate directly malignant B cells through CD40L and IL-4 and favor indirectly the growth of the tumor by stimulating TAM and stromal cells through IL-4; iii) stromal cells are committed towards lymphoid stroma differentiation, in agreement with their contact with TNF-expressing malignant B cells, Tfh, and tumor-associated neutrophils (TAN), and overexpress CCL2 and IL-8 thus recruiting more efficiently TAM and TAN. FL-stromal cells are involved in malignant B-cell recruitment and survival through the release of chemokines, and hedghog (Hh) ligands.

Figure 1

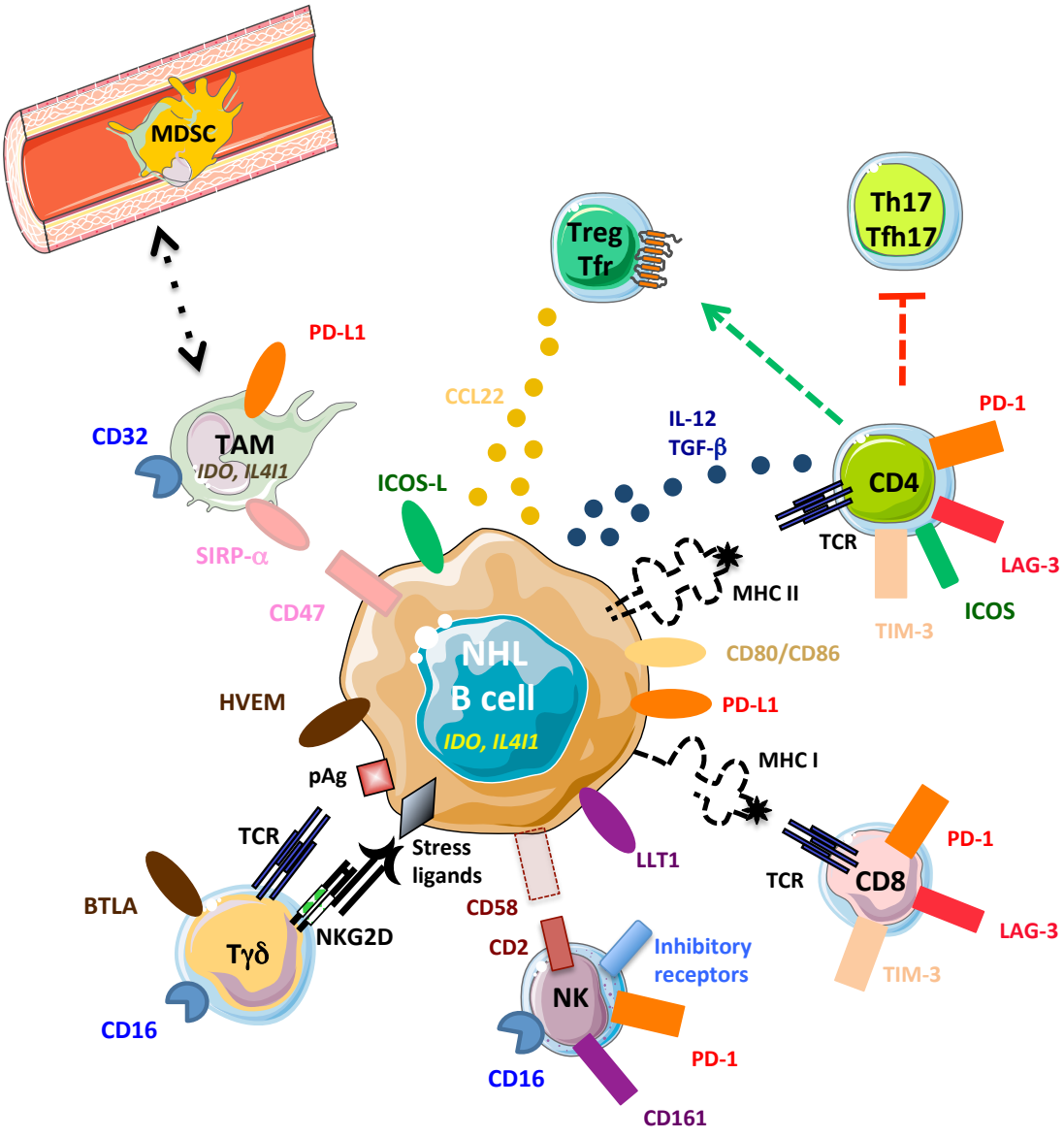
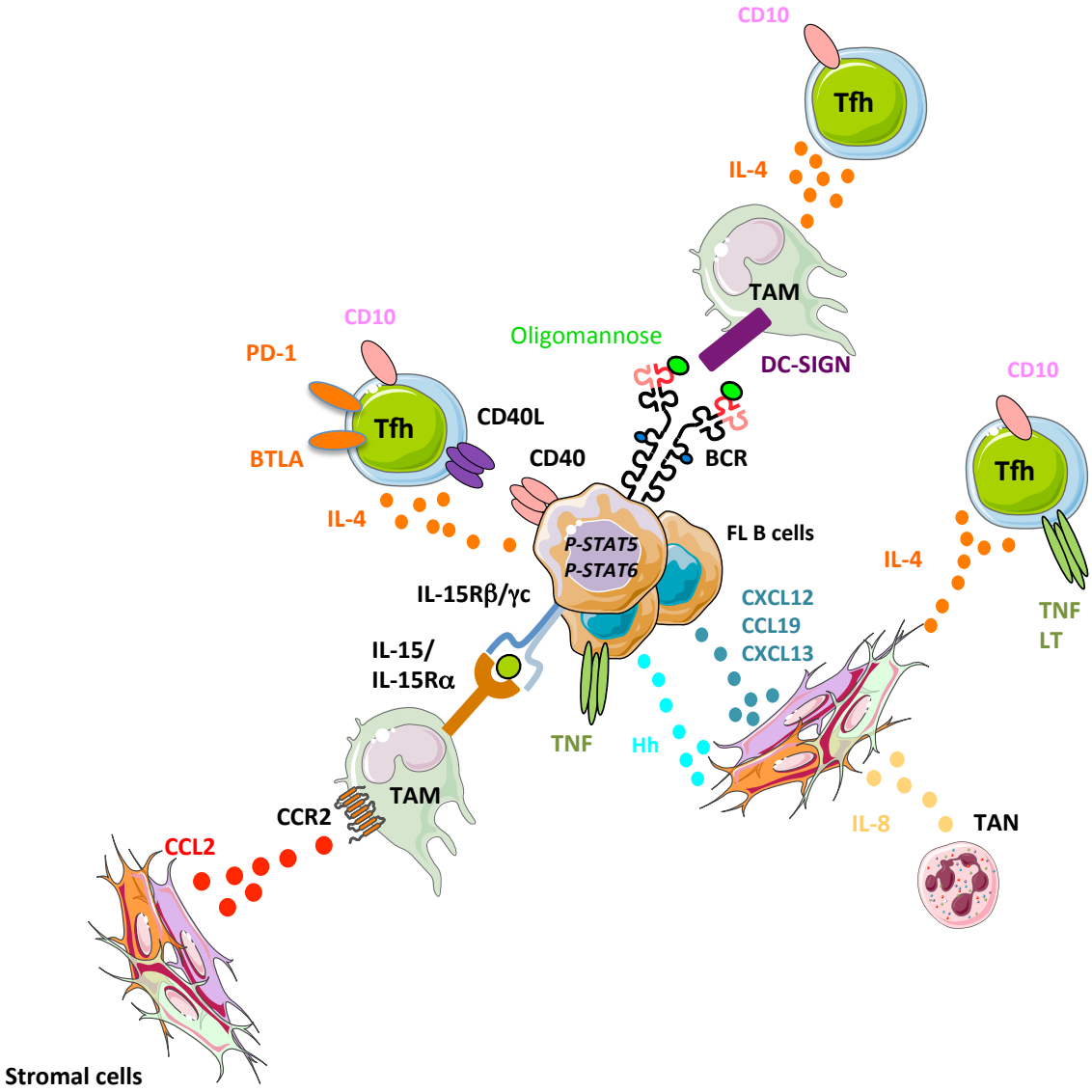

MDSC
Treg
Tfr
Th17
Tfh17
PD-L1
CD32
TAM
IDO, IL4I1
CCL22
IL-12
TGF-β
PD-1
CD4
ICOS-L
TCR
LAG-3
SIRP-α
MHC II
ICOS
TIM-3
CD47
NHL
B cell
IDO, IL4I1
CD80/CD86
HVEM
PD-L1
MHC I
pAg
TCR
Stress
ligands
LLT1
PD-1
TCR
CD8
BTLA
Tγδ
NKG2D
CD58
LAG-3
CD2
Inhibitory
receptors
TIM-3
NK
CD16
PD-1
CD161

Figure 2


CD10
Tfh
IL-4
TAM
Oligomannose
DC-SIGN
CD10
PD-1
Tfh
CD40L
BTLA
CD40
BCR
CD10
Tfh
IL-4
P-STAT5
P-STAT6
FL B cells
IL-4
IL-15Rβ/γc
CXCL12
CCL19
CXCL13
TNF
LT
IL-15/
IL-15Rα
TNF
Hh
TAM
CCR2
IL-8
TAN
CCL2
Stromal cells