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Role of the tumor microenvironment in mature B-cell lymphoid malignancies

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ABSTRACT

The tumor microenvironment is the cellular and molecular environment in which the tumor exists and with which it continuously interacts. In B-cell lymphomas, this microenvironment is intriguing in that it plays critical roles in the regulation of tumor cell survival and proliferation, fostering immune escape as well as the development of treatment resistance. The purpose of this review is to summarize the proceedings of the Second Annual Summit on the Immune Microenvironment in Hematologic Malignancies that took place on September 11-12, 2014 in Dublin, Ireland. We provide a timely overview of the composition and biological relevance of the cellular and molecular microenvironment interface and discuss the role of interactions between the microenvironment and neoplastic cells in a variety of B-cell lymphomas. In addition, we focus on various novel therapeutic strategies that target the tumor microenvironment, including agents that modulate B-cell receptor pathways and immune-checkpoints, chimeric antigen receptor T cells and immunomodulatory agents.

Introduction

Recent advances in the understanding of the pathogenesis of hematologic malignancies have focused attention on the role of the tumor microenvironment. In B-cell lymphomas, the cellular infiltrate intimately associated with the malignant lymphocytes, and the molecules that can be released or trapped within it, may aid tumor cell proliferation and survival as well as escape from immunosurveillance.[1] Recognition of the microenvironment's importance has paved the way for the development of exciting novel strategies that target the microenvironment and its interactions with neoplastic cells. In particular, drugs targeting B-cell receptor (BCR) signaling and programmed death-1 (PD-1) pathways as well as chimeric antigen receptor (CAR) T-cell therapy represent promising advances in lymphoma treatment. The purpose of this review is to summarize the proceedings of the Second Annual Summit on the Role of the Immune Microenvironment in B-cell Lymphomas that took place in Dublin, Ireland on September 11-12, 2014. The manuscript reflects the meeting's structure: the first half is devoted to an overview of the tumor microenvironment in various lymphoma subtypes, and the remaining is a discussion of novel therapeutic approaches targeting the tumor microenviron-

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ment and practical aspects concerning the design and conduct of studies evaluating these agents.

Overview of the microenvironment in B-cell malignancies

The tumor microenvironment of B-cell lymphomas is highly variable with regards to both spatial arrangement and composition of cells, including immune and inflammatory cells, blood and lymphatic vascular networks and the extracellular matrix. The cellular composition of the microenvironment generally mirrors that of the normal tissue at the site of development, the exception being classical Hodgkin lymphoma (see below). Tumor cells retain a degree of dependence on interactions with non-malignant cells and stromal elements of the tumor microenvironment for survival and proliferation.[2] However, tumor cells also use these interactions to generate immunosuppressive mechanisms that promote tumor escape from immune surveillance and lead to disease progression.[2-4] Increasing data indicate a critical role for the tumor microenvironment in mediating treatment resistance.[5] The cellular composition and spatial characteristics of the microenvironment demonstrate significant heterogeneity depending on a number of factors, including the lymphoma subtype. Scott and Gascoyne have proposed three major models that divide up the broad range of tumor microenvironments described in B-cell lymphomas (Figure 1).[2] The first, re-education, is typified by follicular lymphoma (FL), in which malignant cells retain dependence on the microenvironment for survival and proliferation signals; the second, recruitment, is observed in classical Hodgkin lymphoma (cHL) in which the infrequent Reed-Sternberg cells are surrounded by an extensive support milieu of non-malignant cells that is distinct from the composition of normal lymphoid tissue; the third, effacement, is seen in Burkitt lymphoma (BL) and to some extent in diffuse large B-cell lymphoma (DLBCL), whereby genetic aberrations, such as translocation of *MYC*, within the malignant cell lead to autonomous, microenvironment-independent growth and survival.[2] These tumors rely little on the microenvironment, which is sparse when compared to the microenvironment in cHL. Thus, the extent to which different histological subtypes of lymphoid malignancy are susceptible to agents targeting the immune microenvironment is likely to vary depending on the degree to which the tumor cells are dependent on external stimuli for growth or proliferation. In the following section, we provide an overview of the current understanding of the structure, composition and function of the tumor microenvironment in B-cell lymphomas and chronic lymphocytic leukemia (CLL).

Aggressive lymphomas

Diffuse large B-cell lymphoma

DLBCL is the most common type of non-Hodgkin lymphoma and is recognized as a heterogeneous disease with distinct molecular subtypes that are derived from different stages of B-cell differentiation.[6,7] Alizadeh *et al.* first described gene expression profiling to define distinct subtypes of DLBCL: activated B cells and germinal center B cells.[6] Seminal work by the Leukemia/Lymphoma Molecular Profiling Project further described two stromal signatures (termed stromal-1 and -2) in the tumor microenvironment, present in both activated and germinal center subtypes, which were predictive of outcome.[8] Although key genetic lesions may explain some of this disparity, other factors, such as the microenvironment, likely play an important role. The contribution of the tumor microenvironment to the pathogenesis and tumor survival of DLBCL is poorly understood; however, several recent studies have yielded intriguing findings and shed some light on the microenvironment's possible roles. One recent study in DLBCL demonstrated that 29% of cases have mutations or deletions resulting in inactivation of the β_2-microglobulin gene (*B2M*) and 21% feature inactivations in the CD58 gene (*CD58*), two molecules that are critically involved in the immune recognition of tumor cells by circulating T-lymphocytes and natural killer (NK) cells, respectively.[9] The immune escape from these important immune cells (circulating T-lymphocytes and NK cells) implicates the evasion of immune recognition as playing an important role in the pathogenesis of DLBCL. Thus, in the majority of cases of DLBCL these two gene alterations may be co-selected during lymphomagenesis to avoid cytotoxic circulating T-lymphocytes and NK cells.

Many studies have looked at the role of PD-1 and PD-L1, which are expressed in many aggressive B-cell lymphomas and have also been associated with mechanisms of immune evasion.[3,10-12] The MHC class II transactivator CIITA is commonly fused to PD-L1 and PD-L2, which can result in a decrease in HLA-DR expression.[10] A study by Steidl *et al.* looked at rearrangements of *CIITA* in B-cell lymphomas;[10] combined with *PD-L1* copy number gains and translocations independent of *CIITA*, this fusion resulted in T-cell exhaustion and immune escape. In addition, translocations and copy-number gains of *PD-L1/2* appear to be a dominant mechanism of immune escape in primary mediastinal B-cell lymphoma (PMBL).[13-15] Kiyasu *et al.* studied 1253 DLBCL biopsies and found tumor cell, but not microenvironmental, expression of PD-L1 was associated with adverse overall survival, a difference that was present even among the subgroup of patients treated with R-CHOP or similar regimens.[16] Tumor PD-L1 expression was significantly associated with non-germinal center B-cell phenotype.

Other studies have investigated the role of chemokines and cytokines such as CCL22, CCL17, GAL-1 and TGF-β *vis-à-vis* how they recruit and/or retain immunosuppressive cells such as M2 macrophages, regulatory T cells (T_{regs}), and exhausted T cells, and in that way contribute to the pathogenesis of B-cell lymphomas.[2,17,18] Riihijarvi *et al.* found that both CD68 mRNA levels and $CD68^+$ tumor-associated macrophages, detected by immunohistochemistry, were adverse prognostic factors for overall survival among patients treated uniformly with chemotherapy in a prospective clinical trial.[19] In contrast, among patients treated with chemo-immunotherapy, the impact of $CD68^+$ tumor-associated macrophages was reversed, such that patients with high $CD68^+$ tumor-associated macrophages had improved overall survival. This interesting observation led the authors to speculate that rituximab may alter the function of tumor-associated macrophages from having a pro-survival effect to an anti-tumor one.

Mantle cell lymphoma

The molecular hallmark of mantle cell lymphoma (MCL) is the t(11;14) translocation, which results in con-

stitutive expression of cyclin D1, leading to cell cycle deregulation. However, extrinsic microenvironment-derived signals also play a role in the pathogenesis of this disease.[20] MCL is biologically characterized by a tendency toward extranodal dissemination, mediated by attraction and retention through a highly regulated process involving chemokine gradients and adhesion molecules such as VLA-4, CCR7, CXCR5 and CXCR4.[21] Through this mechanism, MCL cells interact with stromal cells such as fibroblasts and macrophages. Adhesion to stromal elements is an important mechanism of chemoresistance, and is likely a reason for the incurability of patients following chemotherapy.[22] Another means by which MCL cells are protected from chemotherapy is through interleukin (IL)-6 secretion, which may be secreted by the MCL cells themselves or by bone marrow stromal cells.[23] IL-6 activates the JAK/STAT3 and PI3K/Akt pathways, known to be key regulators of MCL growth and survival.

Relative to other lymphoma subtypes, the precise composition of the MCL tumor microenvironment is not well characterized. Macrophages have been described in MCL although, in contrast to FL and cHL, systematic evaluation of their prognostic or pathogenic implications is lacking.[24] Studies in small series have suggested that increased numbers of macrophages are associated with aggressive clinical behavior.[25,26] Two studies indicate that MCL cells induce microenvironmental changes to evade the host immune response. Firstly, intratumoral biopsies showed that $CD4^+CD25^+Foxp3^+$ T_{regs} are present in MCL, where they likely contribute to a reduction of anti-tumor cytotoxicity.[18] Secondly, PD-L1 (B7-H1) was shown to be expressed by MCL cell lines, in which it resulted in impaired T-cell proliferation after tumor exposure, inhibited specific anti-tumor T-cell responses and impaired T-cell-mediated tumor cell killing.[27] The negative PI3K regulator PTEN is often inactivated by phosphorylation in MCL.[28] This, along with antigenic stimulation *via* the BCR, resulted in constitutive activation of Syk, Btk and PI3k-Akt, which are critical in MCL disease progression and maintenance.[29] Inhibition of Syk and Btk has been shown to inhibit BCR-mediated adhesion of MCL to bone marrow stromal cells and to increase apoptosis.[30]

Hodgkin lymphoma

The tumor microenvironment in cHL has been extensively studied, with four variant morphological patterns described: nodular sclerosing, mixed cellularity, lymphocyte-rich and lymphocyte-depleted. Neoplastic Hodgkin Reed-Sternberg (HRS) cells account for <5% of the tumor, with the remaining cells comprising B and T cells, eosinophils, neutrophils, mast cells, fibroblasts and macrophages.[31] These cells are attracted by chemokines secreted by HRS cells such as CCL17 (TARC) and CCL12.[32,33] HRS cells also secrete cytokines such as macrophage migration inhibition factor, which induces macrophage M2 polarization,[34] and IL-9, which promotes mast cell differentiation (which in turn results in angiogenesis and fibrosis).[35] Thus, HRS cells both attract and induce the differentiation of immune cells resulting in a tumor microenvironment favorable for tumor cell growth and survival.[36]

The importance of the tumor microenvironment in cHL was illustrated in studies by two independent groups who used gene expression profiling to demonstrate overexpression of genes associated with macrophages in biopsies taken from patients who experienced treatment failure.[37] This tied in neatly with the findings of immunohistochemical studies, in which increased number of $CD68^+$ cells in diagnostic biopsy specimens was prognostic of inferior progression-free survival and disease-specific survival in patients treated with doxorubicin, bleomycin, vinblastine and dacarbazine, independently of established clinical and laboratory parameters.[38] The adverse prognostic impact of CD68 expression on overall survival was validated in another study from Barts Cancer Institute.[39] CD68 is not specific for macrophages, as it stains other myeloid cells, and some fibroblasts.[40] Increased numbers of $CD163^+$ cells [whose expression is restricted to M2 polarized (immunosuppressive) macrophages] has been suggested by some studies to be a superior adverse prognostic marker.[41-43] An interesting recent study showed that patients with Hodgkin lymphoma have higher numbers of circulating myeloid-derived suppressor cells in their peripheral blood than have healthy controls, and that increased levels of $CD34^+$ myeloid-derived suppressor cells were predictive of inferior progression-free survival.[44]

With regard to lymphocyte subsets in the tumor microenvironment, increased numbers of non-follicular B cells are associated with favorable survival, indicating that they likely play an important role in the immunological control of cHL.[39,45,46] Somewhat counter-intuitively, increased numbers of $FOXP3^+$ T_{regs} have been associated with superior progression-free and overall survival.[39,47,48] while increased numbers of granzyme B^+ cytotoxic T cells have the opposite effect on survival.[47,48] Although these findings require validation in larger, prospectively treated cohorts of patients, they suggest that T_{regs} have a contrasting function in cHL compared with solid tumors, such as direct suppression of HRS cells.

Indolent lymphomas

Follicular lymphoma

In FL and mucosal-associated lymphoid tissue (MALT) lymphoma, tumor cells appear to depend heavily on the microenvironment for survival and proliferation.[2] Gene expression profiling of tumor infiltrating lymphocytes (TIL) in FL revealed two immune response signatures which predicted disparate clinical outcomes.[49] Interactions between TIL and tumor cells can result in modulation of the immune response, which can have prognostic implications.[50-54] For example, studies have shown that high numbers of $PD1^+$ TIL are prognostically favorable, while patients with ≤5% $PD1^+$ TIL had a higher risk of histological transformation to DLBCL.[55] In another study from Vancouver, the follicular localization of T_{regs} was found to be an adverse prognostic factor for overall survival and transformation risk.[56]

Tumor-associated macrophages also appear to predict an unfavorable clinical course.[52] Analysis of the gene expression profiles of $CD4^+$ and $CD8^+$ FL TIL revealed altered gene expression that resulted in impaired actin polymerization and immune synapse formation and decreased cytotoxicity and T-cell motility, leading to T-cell exhaustion and immunosuppression.[57-60] This altered gene expression in TIL has prognostic significance with respect to overall survival and time to transformation.[57] In terms of the potential therapeutic implications of these findings in T cells, an interesting study demonstrated that FL cells

with T-cell immunological synapse dysfunction can be repaired with the immunomodulatory agent lenalidomide.[59]

Marginal zone lymphoma

Extranodal marginal zone lymphomas (MZL) of MALT provide a classical illustration of the role of the microenvironment in lymphomagenesis through B-cell antigen stimulation. Chronic infections may provide antigenic stimulation, which results in different manifestations of MZL at various anatomic sites. Examples include gastric MALT and *Helicobacter pylori*,[61] splenic MZL and hepatitis C,[62] ocular adnexal MZL and *Chlamydophilia psittaci*,[63] and cutaneous MZL and *Borrelia*.[64] Eradication of the implicated microorganism leads to lymphoma regression in many cases, supporting antigenic dependence.[65] The occurrence of secondary genetic lesions, in particular t(11;18), has been associated with poor responses to eradication therapy for gastric MALT lymphoma, presumably due to the development of independence from the microenvironment for growth and survival.[66] Although splenic MZL generally has an indolent course, up to one-third of patients experience rapid disease progression. Dense infiltrates of $CD40^+$ cells within the bone marrow correlate with inferior prognosis, likely through interactions with CD40L with surrounding cells in the tumor microenvironment (including mast cells, helper T cells, dendritic cells, macrophages and B cells) resulting in immune cell activation through phosphorylation of STAT3 and resultant secretion of TNF/IL-6 – the net effect of which is the induction of a microenvironment favoring tumor growth and survival.[67]

Chronic lymphocytic leukemia

Studies examining tumor escape in CLL differ as to whether changes in expression of classical and non-classical human leukocyte antigens by tumor cells can modulate the interactions of NK- and T-cell subpopulations with target cells.[68] In CLL, T-cell dysfunction is mediated by expression of inhibitory molecules such as CD200, CD270, PD-L1 and B7-H3 on tumor cells, with predominant influences mediated by PD-L1 expression.[69,70] Expression of these molecules has been linked to a poor prognosis in patients with CLL.[69] Interestingly, reducing expression of these genes in tumor cells can improve T-cell function. In addition, treatment of TIL with lenalidomide has been shown to reverse the signs of T-cell exhaustion and improve T-cell function.[69]

BCL-2 expression[71] has been suggested to be in part controlled by miR-15/16 expression, but alternative microenvironmental interactions may be associated with BCL-2 upregulation and increased cell survival in CLL.[72] Indeed, BCL-2 can be up-regulated by CD40/CD40L interactions, as shown by the increased expression upon culture with soluble CD40L. This interaction may potentially occur in the infiltrated lymphoid tissues and in particular in the proliferation centers where $CD4^+$ T cells can be found in close proximity to leukemic B cells. Moreover, additional studies have shown that co-culture of CLL cells and stromal cells results in up-regulation of BCL-2 expression, thereby providing survival and drug-resistance signals to CLL cells.[73] Investigations into the types of stromal cells that may mediate these interactions show that monocytes contribute to CLL survival and mediate expansion of CLL cells.[74,75] Analyses in murine models show that depleting monocyte levels can decrease CLL burden in the mice.[74] Similarly, the stimulation of surface receptors, including Toll-like receptors[76] and BCR, is able to induce upregulation of BCL-2 and other anti-apoptotic molecules suggesting that a wide array of signals from the microenvironment can indeed be responsible for the regulation of apoptosis. All these signals translate into activation of downstream signaling pathways, including the MAPK and the NF-κB pathways, which contribute to the survival of leukemic cells. ERK is constitutively active in approximately 50% of CLL patients,[77] likely due to the stimulation by anergizing antigenic elements, while SYK and NF-κB

Table 1. Overview of lymphoma subtypes, examples of impact of tumor microenvironment on outcome and novel agents of potential therapeutic relevance.

Lymphoma subtype	Key tumor microenvironment elements, prognostic impact	Therapeutic agents
Hodgkin lymphoma	Increased macrophage gene expression, $CD68^+$ infiltrate (adverse)[37] Increased myeloid derived suppressor cells (adverse)[44] Increased T_{reg} (favorable)[39,47,48] Increased non-follicular B cells (favorable)[39] Increased cytotoxic T cells (adverse)[47,48]	PD-1 inhibitors[153]
Diffuse large B-cell lymphoma	Increased $CD68^+$ TAM and CD68 mRNA (adverse in patients treated with chemotherapy, favorable in patients treated with chemo-immunotherapy)[19] Increased tumor microenvironment PD-L1 expression[16]	Rituximab[19] PD-1 inhibitors[89]
Follicular lymphoma	Immune response signature-1 (favorable)[49] Increased TAM[53] Increased $PD1^+$ TIL (favorable)[55] Intra- or peri-follicular T_{reg} (adverse)[56]	Lenalidomide[59] Lenalidomide and rituximab[132] PD-1 inhibitors[90]
Marginal zone lymphoma	Dense infiltrates of $CD40^+$ cells (adverse)[67]	
Mantle cell lymphoma	Increased TAM associated with aggressive clinical behavior[25,26] Tumor cell adhesion to stromal elements (adverse)[21]	BTK inhibitors[108]
Chronic lymphocytic leukemia	Tumor-stromal interactions[73] Induction of myeloid derived suppressor cells[75] Promotion of BCR signaling and NFκB activation[78]	Lenalidomide[154] BTK inhibitors[106] PI3K inhibitors[121]

PD-1: programmed cell death-1; PD-L1, programmed cell death ligand-1; TAM: tumor associated macrophage; TIL: tumor infiltrating lymphocyte.

are upregulated in virtually all cases of CLL, with many patients having recurrent mutations within the NF-κB pathway[78,79] in addition to induction by the microenvironment.

Novel therapies targeting the microenvironment

The following section focuses on several novel classes of agents that therapeutically exploit the dependence of lymphoma cells on microenvironmental stimuli as part of their mechanism of action.

Checkpoint inhibitors

PD-1 limits the response of activated T cells at sites of infection and prevents autoimmunity.[80,81] Binding of PD-1 by its ligands PD-L1 and PD-L2 produces inhibitory signals that ultimately result in apoptosis of activated T cells, the so-called "immune checkpoint".[82] However, PD-1 is also present on other immune cells including T_{reg}, B and NK cells. Thus, PD-1 blockade enhances anti-tumor cytotoxicity through increased NK-cell killing and T_{reg} suppression.[83,84] Tumor cells are able to exploit the pathway in a similar manner by expressing PD-L1 on TIL.[85] *In vitro* experimental models indicate that PD-L1 expression by tumors results in the impairment of anti-tumor responses.[86] Antibodies targeting the PD-1 axis thus "release the brakes" from effector T cells and promote anti-tumor cytotoxicity.[87] Antibody-dependent cell-mediated cytotoxicity (ADCC) of tumor cells expressing PD-1 or PD-L1 does not appear to be a mechanism of action for these agents, as PD-1/PD-L1 surface expression by tumor cells or tumor microenvironment does not seem to be necessary for their activity.[88] Various agents targeting the PD-1 axis are under development; however, preliminary data

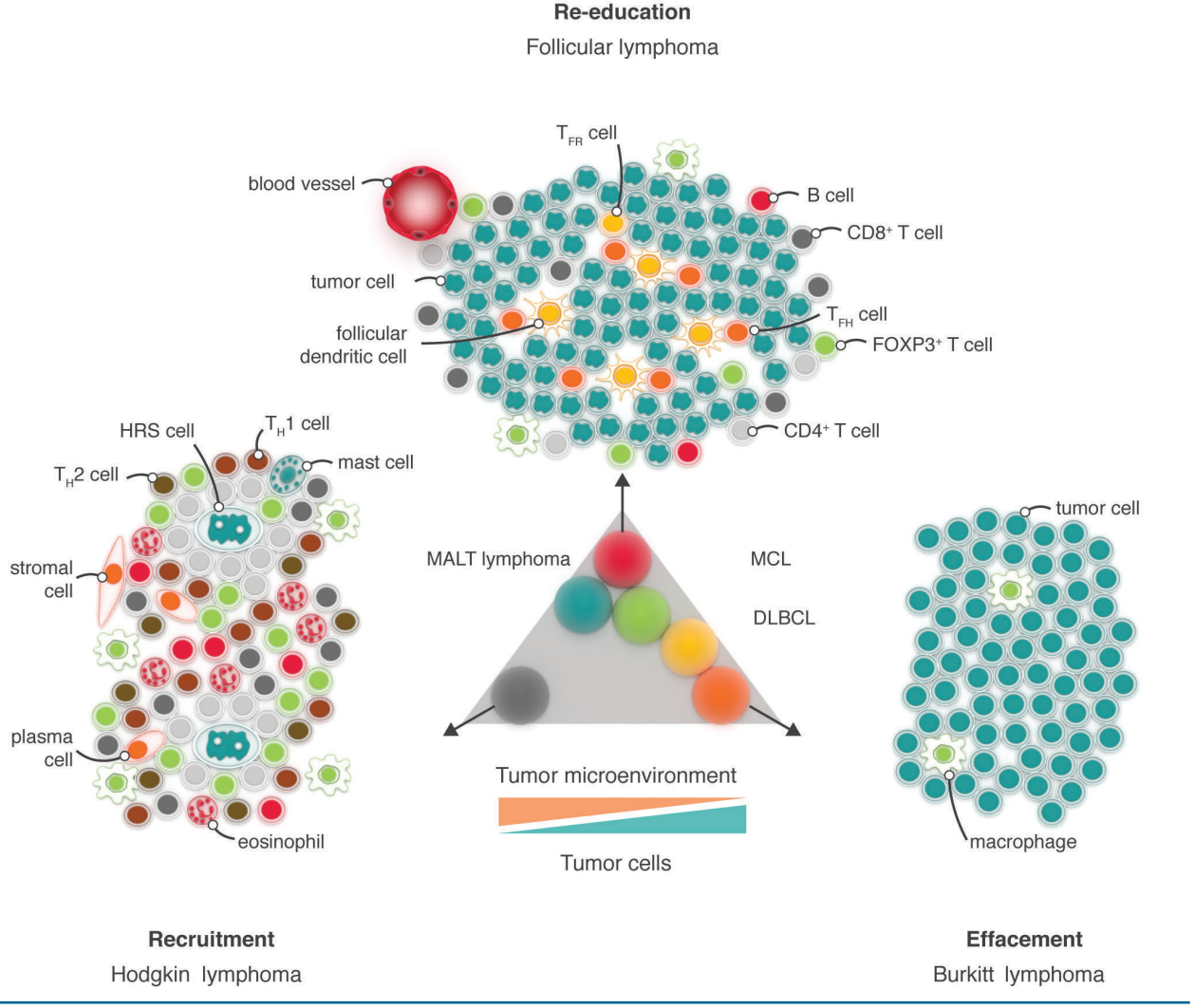

Figure 1. Schematic diagram of the typical microenvironment of the three B-cell lymphoma subtypes that represent the extremes of the spectrum of tumor microenvironment — recruitment, re-education and effacement. These lymphoma subtypes represent the range of tumor cell content, from ~1% in cHL to typically more than 90% in BL. The other B-cell lymphomas fall within this range, as shown for the most common B-cell lymphomas (center). Typically, the ratio of malignant cells to microenvironmental cells increases across the range, from cHL to BL, as shown. DLBCL, diffuse large B-cell lymphoma; FOXP3, forkhead box protein P3; HRS, Hodgkin Reed–Sternberg; MALT, mucosa-associated lymphoid tissue; MCL, mantle cell lymphoma; T_{FH}, follicular T helper; T_H, T helper; T_{FR}, follicular regulatory T. Reproduced from Scott and Gascoyne[2] with permission from Nature Publishing Group.

on three agents are currently available. The investigational agent pidilizumab is a humanized IgG1 monoclonal antibody directed against PD-1, which has been explored in phase II studies in DLBCL[89] and FL.[90] Pidilizumab increased in $CD4^+CD25^+PD\text{-}L1^+$ activated T helper cells and PD-1 ligand-bearing monocytes in a phase II study in DLBCL,[89] and in a phase II study of pidilizumab and rituximab in patients with FL a 41-gene signature representing immune activation correlated with improved progression-free survival.[90] In both studies, pidilizumab was well tolerated and appeared to increase efficacy relative to historic controls. Pembrolizumab (humanized) and nivolumab (fully human), both investigational in hematologic malignancies, are IgG4 antagonistic anti-PD-1 monoclonal antibodies with outstanding activity in heavily pre-treated Hodgkin lymphoma.[91,92] Preliminary results regarding nivolumab show promise in a variety of subtypes of non-Hodgkin lymphomas[93] and phase II studies in multiple histological types are planned or underway.

Chimeric antigen receptor T-cell therapy

Much has been written about the success of investigational anti-CD19 CAR T-cell therapy in relapsed/refractory acute lymphoblastic leukemia, CLL and DLBCL.[94-96] This technology uses gene-modified autologous T cells with antigen specificity for CD19, expressed mainly on the surface of B cells.[97] CD19 represents a near optimal tumor-associated antigen to target, as its restricted expression minimizes off-target toxicity. One of the problems with CAR T-cell therapy is to overcome the immunosuppressive tumor microenvironment that includes M2 polarized macrophages, T_{regs}, and myeloid-derived suppressor cells.[98] Investigators have approached this problem by modifying the CAR T-cell construct number in a number of customized ways, including the incorporation of pro-inflammatory cytokines such as IL-12,[99] expression of dominant negative TGF-β,[100] anti-apoptotic Fas-knockdowns[101] and the expression of survival signals such as Bcl-xl.[102] An alternate approach would be to combine CAR T cells with agents targeting the PD-1 axis to enhance the anti-tumor cytotoxicity.

B-cell receptor pathway inhibitors

B cells depend on signals mediated through the BCR to govern a variety of cellular processes including proliferation, apoptosis and differentiation.[103] Deregulation of the BCR pathway is thought to be central to the pathogenesis of many B-cell lymphomas.[104] The BCR signaling cascade involves numerous tyrosine kinases including Btk, Syk and PI3K, and small molecule inhibitors targeting these kinases have been developed.

Ibrutinib is a selective, small molecule that irreversibly binds to Btk.[105] Ibrutinib has excellent activity in CLL,[106,107] MCL[108] and Waldenström macroglublinemia[109] and has gained regulatory approval for the treatment of relapsed or refractory patients with these diseases and also for first-line therapy in patients with del(17p) CLL. Although the mechanism of action of ibrutinib involves direct effects on malignant B cells, including induction of apoptosis and disruption of cell adhesion and migration,[110] the effects on the tumor microenvironment are also important. Btk regulates NK cell function in response to antigen presentation.[111] However, ibrutinib also inhibits Itk, which is involved in NK cell effector function following FcR-mediated engagement.[112] Interestingly, while some preclinical studies have shown that ibrutinib may antagonize ADCC induced by anti-CD20 monoclonal antibodies such as rituximab, in the clinical setting ibrutinib in combination with rituximab is highly active.[113,114] More selective Btk inhibitors that spare Itk do not appear to have the same antagonism and may prove more effective in combinations. Through Itk inhibition, ibrutinib also influences T-cell polarization toward type 1 T helper cells and effector T cells.[115] Preclinical work by Levy *et al.* at Stanford also suggests that ibrutinib potently enhances immunological tumor control when co-administered with a TLR9 agonist through stimulation of antigen-presenting cells in the tumor microenvironment.[116] The same group also described how ibrutinib enhanced the T-cell anti-tumor activity of PD-L1 inhibitors, a finding with clear implications for combination studies.[117] Btk plays a role in polarizing macrophages to an M1 (inflammatory) phenotype; as mice deficient in Btk are skewed towards M2 (immunosuppressive) polarization, which suggests a theoretical potential for ibrutinib to induce an unhelpful change in the microenvironment.[118] However, we are unaware of data regarding macrophage polarization in ibrutinib-treated patients.

Several PI3K inhibitors with various isoform specificities are in development. The most advanced, idelalisib, is a selective inhibitor of the p110δ isoform of PI3K. It has demonstrated excellent clinical activity in patients with relapsed/refractory CLL/small lymphocytic lymphoma and FL, indications for which it has gained approval from both the Food and Drug Administration (FDA) and the European Medicines Agency (EMA).[119-121] PI3Kδ is expressed by both normal and malignant lymphoid cells, and PI3k inhibition by idelalisib *in vitro* leads to induction of apoptosis.[122] Like ibrutinib, idelalisib interferes with pro-survival microenvironment-derived signals, chemotaxis and adhesion.[123,124] Its antagonism of ADCC induced by anti-CD20 monoclonal antibodies is weaker that that of ibrutinib *in vitro*.[125] Idelalisib does not appear cytotoxic to T-cell subsets;[126] however, the investigational dual PI3K p110γ and p110δ inhibitor duvelisib (IPI-145) reduces the viability of T and NK cells and impairs T-cell production of pro-inflammatory cytokines.[127]

Immunomodulatory drugs

Immunomodulatory drugs exert pleiotropic effects both directly on lymphoma cells and on the immune microenvironment. Lenalidomide (FDA-approved for multiple myeloma and relapsed MCL) has activity in a range of lymphoma subtypes both as a single agent[128-131] and in combination with rituximab, particularly in MCL and FL.[132-137] The molecular mechanism of action of lenalidomide has only recently been described in detail. Immunomodulatory drugs bind to the E3 ubiquitin ligase cereblon (CRBN), which is re-directed by lenalidomide to induce proteosomal degradation of the transcription factors Ikaros (IKZF1) and Aiolos (IKZF3).[138-140] These transcription factors provide pro-survival signals for tumor cells and suppress IL-2 production. The binding of immunomodulatory drugs to CRBN therefore blocks survival signals to tumor cells and leads to increased IL-2 production and enhancement of T-cell co-stimulation.[138] Furthermore, lenalidomide induces type 1 T helper cell polarization,[141] reduces T_{reg} cells, increases antigen presentation to effector T-cell populations,[142] repairs the immune synapse between tumor cells and cytotoxic T cells,[69] restores impaired T-cell motility and interferes with com-

munication between endothelial and tumor cells, reducing neoangiogenesis.[143] Lenalidomide also induces a change in the tumor microenvironment from an M2 macrophage immunosuppressive state to a pro-inflammatory state through polarization of macrophages toward an M1 phenotype.[144] Lenalidomide augments the ADCC of anti-CD20 monoclonal antibodies[145,146] and lowers the activation threshold of NK cells.[147] The multitude of mechanisms by which lenalidomide is able to alter the tumor microenvironment into a hostile one for lymphoma provides a satisfactory explanation for the activity observed in the clinic – an excellent illustration of the potential benefits of targeting the lymphoma cell niche.

Future directions

Novel combinations

It is unlikely that any one agent or modulator of a single pathway will prove successful in inhibiting tumor cell survival over the long-term in B-cell lymphoproliferative diseases. Effective curative strategies will likely require optimal synergistic combinations of effective agents. However, the large number of possible combinations, limited resources and paucity of patients for clinical trials make it an imperative to prioritize and develop those combinations that are most likely to be curative. Designing logical combinations with strong pre-clinical rationales is, therefore, a priority of translational research in hematologic malignancies. Strategies that include the targeting of various steps of the cancer-immunity cycle[148] will be imperative. For example, drugs targeting the PD-1 axis enhance the host anti-tumor response and may be logically used in combination with many of the aforementioned novel agents.[148] Furthermore, "precision immunology" should consider the immunological milieu of both host and tumor. For example, highly immunogenic tumors (such as cHL) may benefit from rational strategies that include immunostimulatory combinations such as PD-1/PD-L1 inhibitors plus T-cell priming treatments.[149] In contrast, immunologically inert lymphomas may be better approached with strategies such as CAR T cells in combination with agents such as monoclonal antibodies.[150]

Caution in developing such combination studies is required and vigilant monitoring for clinical or laboratory adverse events is essential. Two studies using the combination of lenalidomide, rituximab and idelalisib in relapsed/refractory FL were recently terminated due to an unexpected frequency and severity of hepatotoxicity, including two deaths.[151,152] These episodes highlight the need to incorporate correlative studies into all multi-agent investigational protocols to survey for unexpected toxicities as well as to understand tumor biology and the reasons for treatment resistance better.

Monitoring the microenvironment during therapy

Although researchers typically obtain a snapshot of the microenvironment at the time of diagnostic biopsy, the development of processes that enable dynamic assessment is important. Although tumors with a circulating phase, such as CLL, are comparatively easy to assess at serial time-points from blood samples, obtaining biopsies during treatment poses major logistic challenges in most patients with lymphoma. To address this challenge, novel strategies that can assess circulating tumor DNA and mutational analyses in the peripheral blood are welcomed and should be incorporated in future studies aimed at developing therapies directed at the microenvironment.

Conclusion

Improved understanding of tumor biology and the role of the tumor microenvironment has led to advances in the diagnosis, classification, prognostication and novel treatment of patients with hematologic malignancies. In particular, translational research leading to drugs that target the interaction between the tumor microenvironment and malignant cells has provided many promising new approaches to cancer therapy. Ongoing dynamic and correlative studies of tumor biology and the contribution of the tumor microenvironment in the context of novel drug development should be encouraged to identify optimal therapies for various lymphomas and improve the curability of these diseases.

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