Critically dysregulated signaling pathways and clinical utility of the pathway biomarkers in lymphoid malignancies

Rui-Fang Sun a,*, Qian-Qian Yu a, Ken H. Young b,**

a Tumor Biobank, Department of Pathology, Shanxi Cancer Hospital, Taiyuan, Shanxi 030013, China
b Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, TX 77025, USA

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Abstract

Accumulating evidence confirmed that many dysregulated signaling pathways and aberrant genetic alterations contribute to the oncogenesis and heterogeneity of lymphoid malignancies. Therapeutically targeting dysregulating signaling pathways and their hidden oncogenic biomarkers are becoming available, but did not show desired therapeutic effect in current clinical practice. It is meaningful to further understand the underlying mechanisms of the dysregulated signaling pathways and to address the potential utility of pathway-related biomarkers. To precisely identify the dysregulation of signaling pathways and the “driver” oncogenic biomarkers, as well as to develop reliable and reproducible risk-stratification based on biomarkers will be challenging. Nevertheless, pathway-based targeted therapy will raise the hope to improve the outcomes of the patients with lymphoid malignancies, especially with aggressive types, and the efficient utility of pathway-related biomarkers in diagnosis, prognosis, prediction of lymphoid malignancies may also be able to power precision medicine.

Keywords: Lymphoma; Signaling pathway; Biomarker; Therapeutic target

Introduction

Lymphoid malignancies are known for a wide variety of types and molecular and clinical heterogeneity. Increasing evidence supported that many dysregulated oncogenic signaling pathways and aberrant genetic alterations have contributed to the oncogenesis and heterogeneity.1,2 The most frequently dysregulated signaling pathways involved in lymphoid malignancies include B-cell receptor (BCR) pathway, nuclear factor-kappa B (NF-κB) pathway, phosphoinositide-3-kinase/v-akt murine thymoma viral oncogene homolog/mechanistic target of rapamycin (PI3K/AKT/mTOR) pathway, the Janus kinase/signal transducer and activator
of transcription (JAK/STAT) pathway, apoptosis pathway, and programmed death-1/programmed death-ligands (PD-1/PD-Ls) pathway.

In the current era of precision medicine, therapeutically targeting dysregulated signaling pathways and their hidden oncogenic biomarkers are becoming hot topics in the field of cancer research and translational medicine worldwide. Meanwhile, progress has been made in risk-stratification of patients based on the targeted biomarkers, accordingly providing optimal intervention for different risk groups. Many preclinical and clinical trials demonstrated that targeted therapies have clinical activity against a broad spectrum of lymphoid malignancies.3,4

In this review, to comprehensively understand the detailed mechanisms underlying the development of lymphoid malignancies and the potential of targeted therapy, we summarized several key dysregulated signaling pathways involved in oncogenesis and heterogeneity of lymphoid malignancies. The utility of pathway-related biomarkers for diagnostic, predictive, and therapeutic usage is also included.

**BCR signaling pathway**

BCR is a transmembrane receptor whose membrane-bound immunoglobulin can bind to extracellular antigen. Correspondingly, immunoglobulin-linked heterodimer of cluster of differentiation (CD) 79A/CD79B can deliver the antigen stimulatory signals from outside to inside the cell. Following a series of molecules activation, BCR signaling and its downstream signaling cascades consequently control precise function of normal B cells.5

In the three BCR signaling pathways (Fig. 1), the chronic active BCR signaling pathway is classical and antigen-dependent. With the antigen-mediated BCR clustering towards cell membrane, the cytoplasmic tail of BCR, especially immune receptor tyrosine-based activation motifs (ITAM) domain of CD79A and CD79B, becomes phosphorylated by Src family members. Being subsequently recruited and activated by phosphorylated ITAM, spleen associated tyrosine kinase (SYK) thereby activates downstream signals including PI3K/AKT/mTOR signaling, mitogen-activated protein kinase (MAPK) signaling, NF-κB signaling, and nuclear factor of activated T cells (NF-AT) signaling through phosphorylating bruton tyrosine kinase (BTK) and B-cell linker (BLNK). As linker molecules, AKT and BTK are key components to deliver multiple signals.2,6

The tonic BCR signaling pathway and autonomous BCR signaling pathway exist, depending on the interaction between BCR and Lyn/SYK or the two neighboring BCRs rather than external antigenic stimulation.6

Normal BCR signaling has been proven to be functional in B-cell proliferation, survival, apoptosis, and differentiation; aberrantly activated BCR pathway is related to oncogenesis of several types of B-cell hematologic malignancies, especially in chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), active B-cell-like diffuse large B-cell lymphoma (ABC-DLBCL), and mucosa associated lymphoid tissue (MALT) lymphoma.2,7 Furthermore, several key components in these pathways are potential therapeutic targets or diagnostic biomarkers.

**CD79A/B**

CD79A, a transmembrane protein with a cytoplasmic ITAM, forms a CD79A/B heterodimer with CD79B, which is required for the BCR aggregation to induce signal-initiating. In about 20% of ABC-DLBCL cases, CD79A and CD79B mutations can be observed, suggesting their oncogenic roles in dysregulation of BCR signaling pathway in specific ABC subtype.7

**BTK**

BTK is fundamental to the function of BCR signaling pathway and its downstream signaling. As a member of Tec family, BTK is by far the most studied cytoplasmic tyrosine kinase. It is restrictedly expressed in B cells and plays an important role in the differentiation and activation of B cells.8 It is also related to immune function, transcription regulation, and apoptosis modulation due to its function in Toll-like receptor (TLR) pathway and cytokine receptor signaling pathway.9 In BCR signaling pathway, BTK is responsible for receiving signals from SYK and transducing signals to initiate downstream signaling pathway.10

Given the key function of BTK in BCR pathway and downstream NF-κB pathway, an inhibitor against BTK, ibrutinib, showed encouraging efficacy on patients with untreated and relapsed/refractory CLL, ABC-DLBCL, follicular lymphoma (FL), mantle cell lymphoma (MCL), marginal zone lymphoma (MZL), and lymphoplasmacytic lymphoma (LPL). The inhibition against BTK induces downstream kinase inactivation and cell apoptosis through binding to BTK at the C481 residue irreversibly.11,12
NF-κB pathway

NF-κB pathway is one of the most common signaling pathways. In the classical NF-κB pathway that is downstream of BCR and some other pathways, caspase recruitment domain family, member 11/mucosa-associated lymphoid tissue lymphoma translocation protein I/B-cell leukemia/lymphoma 10 (CARD11/MALT1/BCL10) complex is specific for IkB kinase (IKK) phosphorylation which directly activates IkBz. Subsequently, following activation by active IkBz, RelA/p50 heterodimers translocate into nucleus, executing many functions such as cell survival/anti-apoptosis, proliferation, inflammation, and innate immunity (Fig. 1).13–15

In the alternative NF-κB pathway, preferentially, the extracellular initiator is B-cell activating factor receptor (BAFF-R), and the activator of IKK complex that includes two IKKz is NF-κB-inducing kinase (NIK). Additionally, the heterodimer RelB/p50 and RelB/p52 are the major types of dimer. The final effector function of alternative NF-κB pathway mainly involves in lymphoid organogenesis, adaptive immunity, anti-inflammatory properties, and B cell maturation.15,16

These two NF-κB signaling pathways can be seen constitutively activated in almost all types of lymphoma. In ABC-DLBCL, MALT, Hodgkin's lymphoma (HL), Burkitt lymphoma (BL), and some other lymphomas, constitutively activated NF-κB signaling pathway may mostly or partly result in the oncogenic
events, mediated by specific mutation of CARD11, CD79A/B, and myeloid differentiation primary response 88 (MYD88), or chromosomal translocations and Epstein–Barr virus (EBV) infections.7,17–19

The integrated mediators of NF-κB signaling cascades include MYD88, p50, p52, c-Rel, CD30, and CD40, etc.

***MYD88***

MYD88 participates in the activation of both NF-κB and JAK/STAT signaling pathways through TLR/interleukin-1 (IL-1) mediation.20 MYD88L265P mutations have not only been identified as the hallmark of CLL and LPL/Waldenstrom's macroglobulinemia (WM), but also have been found to be related to poor outcome of a subset of DLBCL patients.19 Therefore, it is reasonable to believe that patients with MYD88L265P mutations and dysregulated NF-κB pathways may benefit from drugs inhibiting interleukin-1 receptor associated kinase 4 (IRAK4) that is downstream of MYD88, or interrupting NF-κB pathway, TLR/IL-1 pathway, and other related pathways.20,21

***CD30***

CD30, a transmembrane cell-surface member of the tumor necrosis factor receptor superfamily, is one of the activators of the NF-κB pathway, and is highly restrictedly expressed in tumor cells in classical HL (cHL), anaplastic large cell lymphoma (ALCL), and a subset of DLBCL and EBV-driven lymphoproliferative disorders.22 In clinical practice, CD30 was mainly used as a valuable diagnostic biomarker to diagnose cHL and ALCL. It was also showed in a study that soluble CD30 in serum could predict poor outcomes and disease progression in CD30-positive lymphomas. Apart from as a diagnostic and predictive biomarker, CD30 has been utilized as a target to develop inhibitor agents, typically like brentuximab vedotin, to treat lymphomas with CD30 high expression, such as HL and ALCL.22

***PI3Ks***

PI3K family includes three classes of kinase on the basis of their different structures and functions. Isoforms p110α, p110β, p110δ, and p110γ belong to Class I and are more frequently related to cancer. Among them, p110δ and p110γ are related closely to B-cell, T-cell, and natural killer (NK) cell development, proliferation, cytokine secretion, and other cellular functions.30,31 Therapeutically, as a representative inhibitor of p110δ isofrom, idelalisib has exhibited clinical activity in relapsed CLL, MCL, FL, and DLBCL. In addition to its direct inhibitory effect on p110δ, the efficacy of idelalisib may also be due to the indirect regulation of cytokines and chemokines secretion in the microenvironment. Other inhibitors, such as buparlisib against pan class-I PI3K and duvelisib against p110δ/p110γ isoform, also have showed efficacy in both T- and B-cell lymphomas.34,35

***AKT and PTEN***

AKT and PTEN act as positive and negative mediator of downstream PI3K pathway, respectively, regulating biological processes like cell survival, growth, proliferation, and angiogenesis.36 Owing to some alterations at genetic and epigenetic level, as well as protein–protein interaction, loss-of-function of tumor suppressor PTEN protein may cause the increase of AKT and mTOR activity, which promotes tumor growth and other pathological changes. GCB-DLBCL and MCL are the major lymphoma types involved in functional deficiency of PTEN.37,38

Since the inactivation of PTEN is closely associated with the abnormal activation of PI3K/AKT/mTOR
pathway, therapeutically targeting PI3K/AKT/mTOR pathway to treat malignancies with PTEN deficiency may be rational.\textsuperscript{37,38,41} For instance, MK-2206 as a second-generation AKT inhibitor has been used in patients with relapsed/refractory lymphoma in clinical trials.\textsuperscript{42} Pan-PI3K inhibitor like buparlisib, p110\(\alpha\)-specific PI3K inhibitor like idelalisib, mTOR inhibitor like everolimus, are all in clinical evaluation.\textsuperscript{33,43,44} Additionally, the immunohistochemical expression of phosphorylated-AKT protein can be used to assess outcome or treatment response in some lymphoid malignancies.\textsuperscript{39}

mTOR

Serine/threonine-protein kinase mTOR is a key protein kinase in the PI3K/AKT/mTOR signaling pathway which is closely related to cellular metabolism, survival, and growth through targeting several types of proteins. mTOR is the structural unit for the function of mTORC1 and mTORC2. After receiving phosphorylation signals from upstream AKT, activated mTORC1 and mTORC2 execute their corresponding function, positively regulating mRNA translation, cell survival, cytoskeleton organization, and negatively regulating autophagy, respectively.\textsuperscript{23,24}

Several mTORC1 inhibitors have shown preclinical or clinical efficacy in DLBCL, HL, MCL, FL, CLL/SLL, and even T-cell lymphomas. Temsirolimus and everolimus are the typical mTORC1 inhibitors. They are also expected to have good efficacy with minimized toxicity, either alone or in combination with rituximab.\textsuperscript{43,45,46}

Apoptosis signaling pathway

Apoptosis is a genetically programmed process of cell death and is essential for organism development and environmental homeostasis, which involves many complex molecular mechanisms. There are at least two distinct but interconnected pathways in the process of apoptosis: extrinsic and intrinsic apoptosis pathways (Fig. 1).

Extrinsic apoptotic pathway is initiated when ligands bind to a specific subset of death receptors, such as Fas and tumor necrosis factor receptor 1 (TNFR1). Fas firstly binds to Fas associated via death domain (FADD) and subsequently unites with caspase 8 to form a death inducing signaling complex, promoting apoptosis downstream caspase 3 and caspase 7 directly, or activating caspase cascades via mitochondrial-dependent pathway.\textsuperscript{47,48} Compared with Fas-mediated way, apoptosis mediated by TNFR1 more likely integrates complex TNFRSF1A associated via death domain (TRADD) (death domain-containing proteins like TRADD) and TNF receptor associated factor 2 (TRAF2), inducing cell death or activating NF-\(\kappa\)B function through IKK recruitment.\textsuperscript{49,50}

In the intrinsic apoptosis pathway, apoptosis is triggered immediately more frequently owing to oncogenic stress, DNA damage and hypoxia. The signals in this pathway are transduced and controlled through interacting with p53, DNA checkpoint proteins, and BCL2 family members located in their upstream or downstream pathways, thereby finally activating caspase 3 and caspase 7 to produce effects like extrinsic pathway.\textsuperscript{47,51}

While normal apoptosis pathway is disturbed by multiple cellular and extracellular factors, abnormal proliferation finally results in the oncogenesis of hematologic malignancies. Meaningfully, it is worth exploring the diagnostic, predictive, and therapeutic values of BCL2, Myc, p53 and other molecules involved in apoptosis pathways.

BCL2

BCL2 is one of the members of BCL2 family that programmatically control cell death through anti-apoptotic and pro-apoptotic function. BCL2 suppresses apoptotic death in combination with caspases and other BCL2 family members.\textsuperscript{52,53}

Owing to its translocation with juxtaposed immunoglobulin heavy locus (IgH), t(14; 18)/IgH/BCL2 is a molecular hallmark of FL and can be also detected in some GCB-DLBCL.\textsuperscript{54,55} By contrast, in ABC-DLBCL subtype, BCL2 is more often amplified. Apart from its diagnostic value in lymphoma, both translocation and overexpression of BCL2 can be used to predict aggressive clinical features and adverse outcome in some types of lymphoma.\textsuperscript{40,50}

As for the therapeutic implication, BCL2 appears suitable to develop its inhibitors based on the special structure of BCL2 homology domain 3 (BH3) that can be mimicked through binding to BCL2 or BCL-XL.\textsuperscript{57} Obatoclax and venetoclax (ABT199) are BCL2 inhibitors which can be used for the treatment of relapsed/refractory HL and previously untreated FL while in combination with rituximab through restoring normal apoptosis.\textsuperscript{58–60}

p53

p53, a well-known nuclear transcription factor and tumor suppressor protein, targets a broad spectrum of
functional genes regulating physiological cellular functions and mediates cell apoptosis and growth arrest with the interaction with BCL2 family members and other molecules. As a result, p53 inactivation or the dysregulation of p53-involved signaling pathway has been implicated to be oncogenic event for cancers, including lymphomas.

Theoretically speaking, it seems rational to restore tumor suppressor function of p53, to interrupt mouse double minute-2 homolog (MDM2)-p53 interaction, and to reactivate p53-mediated apoptosis pathways for the development of therapeutics against p53-related malignancies. For example, as a MDM2 inhibitor, APG-115 is such an agent which showed antitumor activities in multiple human cancer xenograft models. It may be able to show activity and safety in human cancer.

**Myc**

Myc is well known as a representative pleiotropic transcription factor and an oncogene protein, targeting numerous genes and regulating multiple processes of cell biology and oncology. Myc has also been associated with oncogenesis, malignant transformation, and aggressive clinical features in many aggressive cancers including aggressive lymphomas. In BL and a subset of DLBCL, chromosomal translocation of MYC like t(8; 14)(q24; q32) is considered as typical oncogenic event and aggressive feature. MYC translocation accompanied by BCL2 or BCL6 is termed as double-hit or triple-hit lymphoma with relatively unfavorable prognosis. A similar situation also occurs in the double-expression lymphoma with Myc and BCL2 co-overexpression. Therefore, both MYC translocation and Myc overexpression have reference value for diagnosis and prognosis of lymphoma in clinical practice.

As for the development of Myc targeting therapeutics, JQ1, a type of small molecule inhibitor against bromodomain-containing protein 4 (BRD4), showed encouraging efficacy in patients with hematologic or some other Myc-driven malignancies through down-regulating Myc function and expression. Another Myc inhibitor against bromodomain and extra-terminal domain (BET)/BRD4 is AZD5153, which showed activity for hematologic malignancies by transcriptionally affecting Myc, E2 promoter binding factor (E2F), and mTOR. Other strategies for developing Myc-targeted therapeutics may be established in reducing Myc/Myc associated factor X (MAX) heterodimerization and DNA binding, affecting MYC-associated chromatin modification, targeting cell cycle kinases, and interfering with its downstream target genes.

**PD-1/PD-Ls signaling pathway**

As a pair of co-inhibitory molecules, normal PD-1 and PD-Ls are employed by immune system to balance immune function. Normally, PD-1 negatively regulates effector T-cell functions with the inhibition of T-cell receptor (TCR)/CD3/CD28 signaling mediated by Src homology region 2 domain-containing phosphatase-1/2 (SHP-1/2) (Fig. 2). However, under the triggering of genetic alteration, virus infection, or other conditions in the pathological process, overexpressed PD-Ls in tumor cells and infiltrating cells lead to exhaustion of effector T cell and final immune escape. Such an immune escape signaling pathway contributes to oncogenesis, tumor aggression and metastasis in many types of malignancies.

Many other immune checkpoint pathways and molecules involved in lymphoma can also function like PD-1/PD-Ls signaling. Such as cytotoxic T-lymocyte-associated protein 4 (CTLA-4)/CD86/CD80, B and T lymphocyte attenuator (BTLA)/herpes virus entry mediator (HVEM), lymphocyte-activation gene 3 (LAG-3)/major histocompatibility complex class II (MHCI), and T cell immunoglobulin mucin-3 (TIM-3)/galectin-9, which are paired respectively to form co-stimulatory signals and co-inhibitory signals, participate in these pathways. Encouragingly, targeting and blocking PD-1/PD-Ls pathway by monoclonal antibodies has been approved to apply in clinical immunotherapy management for cHL, DLBCL, and FL.
anaplastic lymphoma kinase (ALK)-positive ALC.

In FL and CLL/SLL, PD-L1 overexpressed in infiltrating T-cells in follicles of lymph node or tumor cells of CLL cases. Interestingly, both PD-L1 and PD-L2 overexpression was observed in virus-associated lymphomas.

Importantly, accumulating studies have proved that prediction for patient outcome and treatment response is feasible according to the aberrant expression status of PD-1 and PD-L1. Therefore, detection of PD-1 and PD-L1 immunohistochemically will be useful to guide the selection of lymphoma patients.

Additionally, PD-1 combined with other follicular T-helper cell markers like chemokine (C-X-C motif) ligand 13 (CXCL-13) are the robust diagnostic biomarkers for angioimmunoblastic T-cell lymphoma (AITL) diagnosis.

More excitingly, monoclonal antibodies targeting PD-1, pidilizumab, nivolumab and pembrolizumab, have been approved to be used in hematologic malignancies, including cHL, PMBL, FL, CLL/SLL, particularly in relapsed/refractory patients or in combination with autologous hematopoietic stem cell transplantation.

Some inhibitors against PD-L1, durvalumab, atezolizumab, and avelumab, have also been approved by FDA for the treatment of some solid tumors. Many other agents against PD-1 and PD-L1 are also under investigation or clinical evaluation in patients with solid tumor and hematologic malignancies.
JAK/STAT signaling pathway

JAK/STAT signaling pathway essentially mediates cytokine signaling and growth factor signaling, involving a network of molecules with different functions. Upon cytokine receptors-cytokine binding, JAKs and STATs become active sequentially. After that, phosphorylated STATs are dimerized and thereby transfer to nucleus from cytoplasm, causing transcriptional activation of the target genes and eventually regulating apoptosis, proliferation, angiogenesis, and metastasis, etc. In turn, this pathway is negatively regulated by suppressor of cytokine signaling (SOCS) and cytokine-induced STAT inhibitor (CIS) (Fig. 3).92–94

The dysregulation of JAK/STAT pathway can be seen in lymphomas such as PMBL, cHL, and ABC-DLBCL. The oncogenic events may result from loss-of-function of positive regulator JAK2, STAT3, STAT5, and Jumonji domain-containing protein 2C (JMJD2C), as well as negative regulator SOCS1 and protein tyrosine phosphatase non-receptor type 2 (PTPN2).92–96

Four JAKs (JAK1, JAK2, JAK3, and tyrosine kinase 2) generally involve in hematopoiesis, host defense, and immune response. Several abnormalities of JAKs have been identified as specific signatures in lymphomas. Aberrant amplification of JAK2 and JMJD2C with loss of function of SOCS1 and PTPN2 have been shown in cHL and PMBL; JAK3 mutation in NK T-cell lymphoma and adult T-cell leukemia/lymphoma, JAK2/SEC31A fusion involving t(4; 9)(q21; p24) in cHL, and JAK2/PCMI fusion deriving from t(8; 9)(p22; p24) in T-cell lymphoma, all have been identified.97–100

Inhibitor against JAK2/Fms-like tyrosine kinase 3 (FLT3) fusion, pacritinib (SB1518), has been developed for treatment of relapsed/refractory cHL, FL, and DLBCL, demonstrating clinical safety and efficacy.101 Meanwhile, tofacitinib, JAK3 inhibitor, has therapeutic efficacy for EBV-associated NK- and T-cell lymphoma, probably due to its ability to decrease STAT5 phosphorylation, to inhibit proliferation, and to reduce EBV latency.102

Fig. 3. Illustration of JAK/STAT signaling pathway. AKT: v-akt murine thymoma viral oncogene homolog; BCL: B-cell leukemia/lymphoma; CIS: cytokine-induced STAT inhibitor; Erk: extracellular signal-regulated kinase; JAK: Janus kinase; mTOR: mechanistic target of rapamycin; MEK: methyl ethyl ketone; PI3K: phosphoinositide-3-kinase; SHP: Src homology region 2 domain-containing phosphatase; SOCS: suppressor of cytokine signaling; STAT: signal transducer and activator of transcription; VEGF: vascular endothelial growth factor.
STATs

STATs family consist of seven members: STAT1, STAT2, STAT3, STAT4, STAT5α, STAT5b and STAT6. STAT3 expression increased significantly in ABC-DLBCL and ALCL patients; in the latter, nucleophosmin 1 (NPM-1)/ALK fusion was proposed to be related to increased STAT3. Additionally, STAT3 and STAT5 activation can be observed in cutaneous T-cell lymphoma, and STAT6 mutation occurs in PMBL and FL.

Therapeutically, a STAT3 inhibitor, pyrimethamine, for the treatment of relapsed CLL/SLL, is being estimated in phase I/II clinical trial (Clinical Trials Registration: NCT01066663).

Remarks and conclusion

As noted above, it has been well recognized that complex network of dysregulated signaling pathways and their hidden oncogenic alterations contribute a lot to lymphoma oncogenesis and heterogeneity. In the era of precision medicine, it is imperative to precisely identify the dysregulation of these signaling pathways and the “driver” oncogenic biomarkers; it is meaningful to further understand the underlying mechanisms of the dysregulated signaling pathways and to address the potential utility of pathway-related biomarkers. Table 1 summarized the pathway-related biomarkers and their clinical utilities in lymphoid malignancies.

With the progress of biotechnologies and the application of advanced detection platform, identifying pathway-based biomarkers becomes available, providing good opportunities to utilize these biomarkers reasonably and feasibly. The two most promising utilizations lie in precise diagnosis and treatment. Instead of relying solely on morphologic and histologic features and clinical experience, molecular classification based on specific biomarker has almost become a requirement for diagnosis, risk-stratification, and treatment-guidance in some lymphoid malignancies.

These pathway-based biomarkers may include gene rearrangement, gene amplification, specific tumor-driving mutations, epigenetic alterations, unusual expressions, as well as other abnormal genotypes and phenotypes. Therefore, faced with so many complicate alterations, there are big challenges to utilize these biomarkers reasonably and efficiently.

In biomarkers screening and detecting, reliable and reproducible biomarkers accurately conveying activated oncogenic pathways in patients will be needed. For example, PD-1 and PD-L1 are not only the therapeutically targeted biomarkers but also predictive biomarkers to guide patients who may benefit from immunotherapy. Moreover, PD-1 is useful forAITL diagnosis. In practice, the robustness of PD-1 and PD-L1 detection may depend on the specificity and sensitivity of the antibody, the platform or method used, the status of intra-tumor heterogeneity, and other factors.

Additionally, in front of enormous amount of information triggered by complex techniques, especially by next generation sequencing (NGS), we are confronted with many problems in the discovery and application of new biomarkers. For instance, despite NGS is becoming more and more popular and the cost is getting lower and lower, in clinical practice, a panel sequencing focusing on a limited number of genes is still a better option than whole genome sequencing. Nevertheless, it will be a trend to apply whole genome sequencing and transcriptome sequencing in routine clinical practice in the future with the advances of technology and increased availability. Another focus is that in addition to single specific diagnostic or prognostic biomarker, rational algorithms or some grouped gene signatures are of great value for routine diagnosis, classification, prognosis, prediction, or monitoring in lymphomas. In fact, a lot has been put into practice.

As for precision treatment, many arguments have emerged. Are patients with malignancies matched with drugs for mutations or “pathway” mutations? Why did the current targeted drugs not achieve desired therapeutic effect in clinical practice? How do clinicians choose the best treatment regimens? Single targeted agent alone? Or in combination with multiple pathway inhibitors? We do need to notice and address these issues. It will be difficult to assess the effect of combination regimens using predictive biomarkers from individual patient. Probably a long-time and large population validation is required for the effect of predictive biomarkers and targeted therapies. In addition, the side effects of these targeted therapies in lymphomas are also of great concern. Here, we summarized the side effects of agents targeting pathway-related biomarkers in Table 2.

In conclusion, despite the many problems encountered, pathway-based targeted therapy in combination with traditional chemotherapy, single specific targeted antibody, and immunotherapy will raise the hope for the patients with lymphoid malignancies. The rational and efficient utilization of pathway-related biomarkers in diagnosis, prognosis, prediction, and treatment selection in lymphoid malignancies will power precision medicine.
| Oncogenic biomarkers | Related signaling pathways | Functions | Clinical utilities | Targeted agents | Diseases applied | Detection approaches |
|---------------------|---------------------------|-----------|-------------------|----------------|-----------------|---------------------|
| BTK | BCR, NF-κB signaling pathway | Modulates B-cell development, immune function, transcription, and apoptosis | Therapeutic | BTK inhibitor: Ibrutinib (PCI-32765), GBB-3111, AVL-292 cc-292, M7583, Acalabrutinib (ACP-196) | CLL/SLL, R/R MCL, ABC-DLBCL, FL, MZL, T-cell lymphoma | Sequencing, PCR, IHC |
| MYD88 | NF-κB, IL-1, TLR signaling pathway | Acts as a signal transducer in the IL-1, IL-18, and TLR signaling pathways; involves in innate and adaptive immunity | Diagnostic: *MYD88* L256P mutation for LPL/WM, HCL; prognostic: ABC-DLBCL | TLrs ligands inhibitor: IMO-8400; IRAK4 inhibitor: ND-2158, ND-2110 | ABC-DLBCL with *MYD88* L256P mutation, other malignancies harboring aberrant *MYD88*, LPL/WM | Sequencing, PCR, IHC |
| CD30 | NF-κB pathway | CD30 overexpression in cHL, ALCL | Diagnostic and differential diagnostic: Anti-CD30 antibody: Brentuximab vedotin | | | |
| PI3Ks | PI3K/AKT/mTOR, BCR, TLR/MYD88 pathway | PI3Kα involves in cell growth, proliferation, survival, and morphology; PI3Kγ involves in B-cell, T-cell, and NK cell development, proliferation, migration, and cytokine production | Therapeutic | PI3K inhibitor: SAR 245408 (XL147); PI3Kα/γ inhibitor: Duvelisib (IPI-145, INK1197); PI3Kα inhibitor: Idelalisib (CAL-101, GS-1101), AMG 319, Acalisib (GS-9820) (CAL-120); PI3Kγ inhibitor: Buparlisib (BKM120) | R/R NHL, advanced hematologic malignancies | Sequencing, PCR, IHC |
| AKT | PI3K/AKT/mTOR, BCR, TLR/MYD88 pathway | Regulates cell survival, growth, proliferation, and angiogenesis | Prognostic and predictive: abnormal phosphorylated-AKT expression; Therapeutic | Akt inhibitor: Perifosine (KRX-0401), MK-2206, GSK690693 | Hematologic malignancies, Lymphomas | IHC, Sequencing, PCR |
| mTOR | PI3K/AKT/mTOR, BCR, TLR/MYD88 pathway | Regulates cellular metabolism, survival, growth; mTORC1 regulates mRNA translation, protein synthesis, and autophagy; mTORC2 regulates cell survival and cytoskeleton organization | Therapeutic | mTOR inhibitor: Temsirolimus (CCI-779), Rapamycin (Sirolimus), Ridaforolimus (AP23573, MK-8669); mTORC1/mTORC2 inhibitor: Everolimus (RAD001), AZD2014 | Lymphoma, multiple myeloma, hematologic malignancies | Sequencing, PCR, IHC |
| BCL2 | Apoptosis pathway | Suppresses apoptotic death | Diagnostic: Overexpression and translocation in FL, double-expression or double-hit lymphoma; amplification in ABC-DLBCL | BCL2 inhibitor: Venetoclax (ABT199), Obatoclax Mesylate (GX15-070MS) | FL, DLBCL, R/R lymphoid malignancies | IHC, ISH, PCR, sequencing |
| p53 | Apoptosis pathway | Regulates transcription; regulates cell cycle; induces growth arrest or apoptosis | Therapeutic | MDM2 inhibitor: APG-115, DS-3032 | Advanced solid tumors or lymphomas | IHC, PCR, sequencing |
| Pathway | Involvement | Diagnostic and Prognostic | Therapeutic | Therapeutic Medications |
|---------|-------------|--------------------------|-------------|------------------------|
| Myc Apoptosis pathway | Involves in cell cycle progression, apoptosis and cellular transformation | Overexpression and translocation in BL, double-expression or double-hit lymphoma; | BET inhibitor: JQ1, CPI-0610, AZD5153; Aurora A inhibitor: Alisertib; c-Myc-Max dimerization inhibitor: 10058-F4 | BL, DLBCL, hematologic malignancies |
| PD-1 Immune system pathway, TCR pathway | Negatively regulates effector T-cell functions | Anti-PD-1 antibody: Pidilizumab (CT-011), Nivolumab (BMS-936558, MDX-1106, ONO-4538), Pembrolizumab (lambrolizumab, MK-3475) | Aggressive B-cell lymphomas; advanced malignancies; T-cell or NK-cell lymphomas | IHC, ISH |
| PD-L1 Immune system pathway, TCR pathway | Inhibits T-cell activation and cytokine production upon interaction with PD-1 | Anti-PD-L1 antibody: Durvalumab (MEDI4736), Avelumab (MSB0010718C), Atezolizumab (MPDL3280A) (RG7446) | Lymphomas, solid tumors; Advanced cHL; R/R PTCL | IHC, ISH |
| JAKs JAK/STAT pathway | Involves in cell growth, development, and differentiation; mediates adaptive and innate immunity | JAK2/SEC31A fusion in cHL; JAK2/PCMT1 fusion in T-cell lymphoma; JAK2/FLT3 fusion in cHL, FL, and DLBCL; Aberrant JAK2 and JMJD2C amplification in cHL and PMBL; | JAK3 inhibitor: Tofacitinib (CP-690550); JAK2 inhibitor: Pacritinib (SB1518), Ruxolitinib (INCBO18424) | Therapeutic |
| STATs JAK/STAT pathway | STAT3 involves in cell growth and apoptosis; STAT6 activates transcription, involves in IL-4 signaling, induces anti-apoptotic activity | STAT3 inhibitor: Pyrimethamine, IONIS-STAT3Rx (ISIS 481464) | Relapsed CLL/SLL; lymphomas, advanced cancers | Sequencing, PCR, IHC |
| Biomarker | Therapeutics | Agent | Involved lymphomas | Phase | Common side effects |
|-----------|-------------|-------|---------------------|-------|---------------------|
| BTK       | BTK inhibitor | Ibrutinib (PCI-32765) | Recurrent B-cell lymphoma, CLL/SLL, R/R MCL, ABC-DLBCL, FL, MZL, T-cell lymphoma | Phase 1/2 | Mild diarrhea, nausea, fatigue, upper respiratory tract infections, rash, dyspnea |
| BTK       | BTK inhibitor | BGB-3111 | R/R B-cell malignancies | Phase 1 | Minimal side effects |
| BTK       | BTK inhibitor | Acalabrutinib (ACP-196) | R/R ABC-DLBCL; CLL/SLL; MCL | Phase 1/2 | Comparatively less toxicity, including rash, major bleeding and atrial fibrillation |
| PI3Kδγ | PI3Kδγ inhibitor | Duvelisib (IPI-145, INK1197) | R/R NHL, advanced hematologic malignancies | Phase 1/2 | Tansient cytopenias, febrile neutropenia and pneumonia |
| PI3Kδ | PI3Kδ inhibitor | Idealisib | R/R MCL, FL, SLL, LPL, MZL | Phase 1/2 | Fatigue, diarrhea, nausea, rash, chills, and pyrexia |
| PI3Kγ | PI3Kγ inhibitor | Buparlisib (BKM120) | PCNSL, SCNSL, CLL/SLL | Phase 2 | Neuropsychiatric symptoms such as mood alteration, suicidal ideation, and altered mental status associated with its use |
| PI3Kαδ | PI3Kαδ inhibitor | Copanlisib (Bay 80-6946) | NHL, aggressive B-cell lymphomas | Phase 3 | Hypertension, neutropenia, hyperglycemia, diarrhea, and fatigue |
| AKT       | Akt inhibitor | Perifosine (KRX-0401) | Hematologic malignancies, Lymphomas | Phase 1/2 | Nausea, vomiting, diarrhea, and fatigue |
| Akt1/2/3 | Akt1/2/3 inhibitor | MK-2206 | Relapsed lymphoma, R/R DLBCL | Phase 2 | Dehydration, hyperglycemia, rash and neutropenia |
| mTOR     | mTOR inhibitor | Temsirolimus (CCI-779) | R/R HL; R/R PCNSL; FL, CLL/SLL, R/R MCL | Phase 1/2/4 | Thrombocytopenia |
| mTORC1/mTORC2 | Everolimus (RAD001) | R/R NHL, R/R MCL, R/R cutaneous T-cell lymphoma | Phase 2 | Neutropenia, anemia, and thrombocytopenia in DLBCL |
| BCL2     | BCL-2, BCL-XL | Navitoclax (ABT263) | R/R lymphoid malignancy | Phase 2 | Diarrhea, nausea, vomiting, fatigue and dose-dependent thrombocytopenia |
| BCL2     | BCL-W inhibitor | Navitoclax (ABT199) | NHL, CLL/SLL, MM, R/R NHL | Phase 1/2/3 | Diarrhea, neutropenia, fatigue, upper respiratory tract infection, and cough |
| BCL2     | BCL2 inhibitor Obatoclax Mesylate (GX15-070MS) | R/R HL | Phase 2 | Neurologic toxicity |
| P53      | P53-MDM2 blockade | ALRN-6924 | Advanced solid tumors or lymphomas | Phase 1/2 | GI side effects, fatigue, anemia, and headache |
| Myc      | Aurora A inhibitor | Alisertib | Myc-positive aggressive B-cell lymphomas | Phase 1 | Myelosuppression alopecia, mucositis and fatigue |
| BET      | BET inhibitor | JQ1 | Hematologic malignancies | Preclinical | Pre-clinical development, no serious side effect was reported |
| Syk      | Syk inhibitor | Fostamatinib (R788) | CLL/SLL, DLBCL, MCL, FL, T-cell lymphoma | Phase 1/2 | Fatigue, diabetes, hypoglycemia, and hypertension |
| PD-1     | Anti-PD-1 antibody | Pidilizumab (CT-011) | DLBCL and PMBL after ASCT; Stage III-IV DLBCL | Phase 2 | Mild fatigue, rash, pruritus, diarrhea, and colitis |
| PD-1     | Anti-PD-1 antibody | Nivolumab (BMS-936558, MDX-1106, ONO-4538) | R/R DLBCL, PCNSL, PTL, FL, PTCL | Phase 2 | Mild fatigue, rash, pruritus, diarrhea, and colitis |
| PD-1     | Anti-PD-1 antibody | Pembrolizumab (lambrolizumab, MK-3475) | R/R FL; DLBCL and T-NHL after ASCT; T-cell or NK-cell lymphomas; R/R HL; recurrent PCNSL; R/R PMBL | Phase 2 | Mild fatigue, rash, pruritus, diarrhea, and colitis |
Conflicts of interest

All authors declare no conflicts of interest.

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