Chapter 12
Towards Targeted Therapy of Chronic Lymphocytic Leukemia

Carsten U. Niemann, Jade Jones, and Adrian Wiestner

Abstract The B cell antigen receptor (BCR) and its downstream pathways are pivotal in the pathogenesis of chronic lymphocytic leukemia (CLL). Recently, inhibitors of kinases in the BCR pathway have shown promising clinical activity in CLL. Based upon these results, the treatment paradigm for CLL will likely undergo major changes. The kinases essential for BCR signal transduction, which are emerging as targets for CLL treatment, and the specific inhibitors under development are the focus of this chapter. In particular, the BTK inhibitor ibrutinib and the PI3K inhibitor idelalisib (GS-1101) are two evolving targeted therapies with the most mature clinical data.

Keywords BCR (B cell antigen receptor) • Microenvironment • Targeted therapy • Lymphocytosis • Ibrutinib (PCI-32765) • idelalisib (GS-1101, CAL-101) • Everolimus (RAD001) • Navitoclax (ABT-263) • ABT-199 • Lenalidomide • Dasatinib (BMS-354825) • Fostamatinib (R788) • BTK (Bruton's tyrosine kinase) • SYK (spleen tyrosine kinase) • LYN • PI3K (phosphatidylinositol 3 kinase) • mTOR (mammalian target of rapamycin) • BCL-2

Introduction

Differences in the somatic mutational status of the immunoglobulin loci among B cell chronic lymphocytic leukemia (CLL) patients were revealed almost 20 years ago [1]. Since that time, the B cell antigen receptor (BCR) and the downstream pathways have been thoroughly investigated. In 1999, the groups of Stevenson and Chiorazzi demonstrated the prognostic significance of unmutated vs. mutated

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IGHV (immunoglobulin heavy chain variable gene cluster) genes among CLL patients [2, 3]. A few years later, the concept of stereotyped motifs for the IGHV gene among CLL patients [4] further underscored the significance of the BCR pathway in CLL and pointed towards CLL as a partially antigen-driven disease. Most recently, an autonomous signaling through interaction of the HCDR3 of the heavy chain of the BCR with an internal epitope (FR2) of the BCR in CLL cells was shown [5]. This finding will probably modify the understanding of the pathogenesis of CLL significantly. It may also allow for new treatment targets to be revealed [6]. Along with this growing understanding of the role of the BCR in CLL, the differences in phenotype and sensitivity to different treatments among CLL cells in peripheral blood, bone marrow, and lymph nodes have underscored the impact of the microenvironment on the course of CLL [7]. This is further emphasized by the effect of new targeted therapies for CLL which cause redistribution of CLL cells from lymph nodes to peripheral blood [8]. Furthermore, the corroboration of tissue and lymph node as the proliferating compartment compared to the predominantly resting CLL cells of peripheral blood underscores the significance of the microenvironment in CLL [9]. BCR signaling is likely a pivotal pathway activated in CLL cells in lymphatic tissue as indicated by gene expression profiling of lymph node-derived CLL cells compared to peripheral blood CLL cells from the same patient [10]. Even though the BCR pathway has been well characterized for decades, the intricate interrelation of downstream signaling cascades continues to be extended and modified. Several targets for development of new treatments in CLL have been revealed, along with new achievements in the understanding of BCR signaling [11].

Targets in CLL: Reflections of Normal B Cell Pathways

The molecules in the BCR pathway that are emerging as targets for CLL treatment are outlined in Fig. 12.1. The focus of this chapter is on molecules that are currently targeted in clinical trials or for which there are significant preclinical data. Initially, an overview of the evolving concepts of how B cells interact with the microenvironment and a discussion of different types of BCR signaling is presented.

Initiation of BCR Signaling

The conformational state of BCRs in resting B cells has hitherto not been fully outlined. Moreover, the change in the conformation of the BCR that propagates signaling upon antigen binding is not clearly understood. One model describes the BCRs of resting B cells as inhibited oligomers flowing in the plasma membrane. Upon antigen stimulation, the state of the BCR changes to an open conformation for signal propagation [12]. Another model describes oligomerization upon antigen binding that initiates signaling [13]. The detection of stereotyped BCRs in some subsets of CLL patients [4]

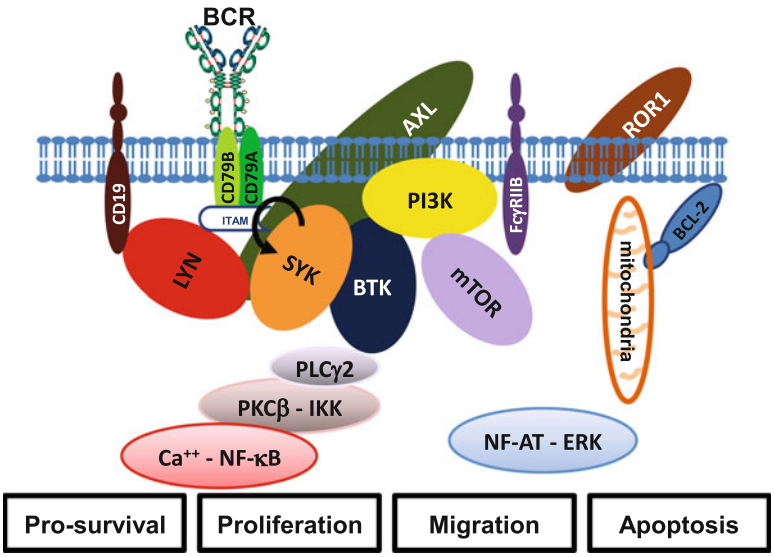

Fig. 12.1 Signaling through the B cell antigen receptor (BCR) with downstream and parallel pathways in chronic lymphocytic leukemia (CLL) outlined. Antigen binding or cell autologous interaction initiates BCR signaling, causing phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) in the cytoplasmic domains of co-receptors CD79A and B. LYN has a double function in initiating and terminating BCR signaling depending on interaction with CD19. Spleen tyrosine kinase (SYK) amplifies the initial signal by further phosphorylation of ITAMs. SYK also activates phosphatidylinositol 3 kinase δ (PI3Kδ) that in turn converts phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-triphosphate (PIP3). Mammalian target of rapamycin (mTOR) relays PI3K activation further to cell cycle regulation. Bruton's tyrosine kinase (BTK) is recruited to the plasma membrane and activates PLCγ2, which in turn generates a set of second messengers to activate protein kinase C β (PKCβ). PKCβ phosphorylates IκB kinase (IKK) to activate NF-κB transcription factors that regulate gene expression of several survival factors. Signaling can be modulated by interaction with BCL-2 family members guarding apoptosis induction. FcγRIIB inhibits BCR signaling upon binding of complement. The downstream effectors can be turned towards the pro-apoptotic NF-AT–ERK pathway, depending on balancing of the signaling cascades. ROR1 signals through the alternative Wnt pathway. Only molecules of relevance for the discussion of evolving targeted therapies in CLL are included. Please see the sections on specific parts of the signaling pathways for further details

has resulted in research to determine the antigenic drive in CLL. Based on affinity to some of the stereotyped immunoglobulins, candidate autoantigens like non-muscle myosin from apoptotic cells [14] and common microbial antigens [15] have been identified as probable drivers for CLL cells [16]. However, a new understanding of dysregulated BCR signaling has been proposed. Dühren-von Minden et al. showed that the HCDR3 region of the BCR binds the FR2 epitope of the same or adjacent BCRs on the same cell [5]. Transfer of the HCDR3 region from both mutated and unmutated as well as stereotyped and non-stereotyped CLL cells to normal B cells conferred the chronic BCR signaling of the CLL cells to the normal B cells. The elucidating of this

new mechanism for autonomous signaling through the BCR and the impact on the understanding of the pathogenesis of CLL await further research [6]. Most importantly, the revealing of a pathway leading to autologous chronic activation of the BCR emphasizes the molecular basis for targeting this pathway to treat CLL.

From BCR Activation to Phosphorylation

Within the cytoplasmic part of the BCR, ITAMs (immunoreceptor tyrosine-based activation motifs) were identified more than 20 years ago as probable intracellular signal generators. ITAMs are specific preserved sequences of the CD79A/B of the BCR complex [17], which are also represented in other pathways of the immune system, like the CD3 components of the T cell receptor. ITAMs were shown to link antigen binding by BCR to intracellular signaling cascades [18]. Binding of antigen to the extracellular part of the BCR confers initiation signals to the phosphorylation of ITAMs in the cytoplasmic part of CD79A/B. Several of the kinases in the BCR pathway bind directly to ITAMs.

ITAMs are also involved in the anergic state of some B cells by monophosphorylation of ITAMs. This mechanism is part of the host's protection against autoreactivity [19]. Anergic B cells are described as being in a condition of unresponsiveness to antigen stimulation induced by chronic autoantigen occupation of a part of the BCRs [20]. Furthermore, mutation of a critical tyrosine residue in the ITAM of CD79B seems to allow abnormally active responses to chronic antigen stimulation through attenuation of LYN signaling in some B cell malignancies [21].

Signal Propagation

Propagation and amplification of BCR signaling, as well as further clustering, is dependent upon downstream signaling through spleen tyrosine kinase (SYK) and LYN [22]. Whereas LYN has a balancing effect on BCR signaling, both initializing and terminating the signaling cascade, SYK amplifies the signal through further phosphorylation of ITAMs at CD79A/B (see Fig. 12.1). Thus, SYK and LYN have been among the first targets for clinical trials of inhibition of BCR pathway kinases in CLL. On the other hand, in resting B cells, a tonic survival signal is relayed through the phosphatidylinositol 3 kinase (PI3K) pathway downstream of the BCR that does not require phosphorylation of ITAMs, SYK, or LYN [13]. It has been suggested that part of the pathogenesis of CLL involves dysregulation of the tonic signaling of resting normal B cells [11].

Balancing BCR Signaling

In normal B cells, FcγRIIB is an inhibitory co-receptor that inhibits BCR signaling in response to complement binding, thus taking part in the balancing of the BCR pathway. The inhibitory effect of activated FcγRIIB is at least in part mediated by interaction with LYN and BCR oligomer formation early in the BCR pathway [23]. These balancing effects may be dysregulated in CLL cells, thus adding support to targeting of LYN [24]. The understanding of the dynamics in the interaction of membrane-bound proteins, properties inherent to the lipid bilayer, and the cytoskeleton is evolving. It appears that FcγRIIB activation, as well as inhibition of Bruton's tyrosine kinase (BTK) and PI3K, affects the mobility of BCRs in the plasma membrane. For BTK and PI3K inhibition, the effect is through inhibition of sphingolipid glucosylation [25]. This may in part explain the effect of BTK and PI3K inhibition on abrogating the BCR signaling in CLL.

The tonic signaling through the BCR of a resting B cell is also dependent on interaction between CD79B and the actin cytoskeleton that restricts the movement of the BCR in the plasma membrane [26]. BTK directly interacts with the actin cytoskeleton during BCR signaling, causing migration of B cells and internalization of the BCR [27]. Thus, targeting BTK may modulate mobility of the BCR in the plasma membrane by interaction with both actin and sphingolipid synthesis, in addition to inhibiting downstream activation signals from the BCR.

Internalization of BCR was initially seen as a way of extinguishing the antigen activation of B cells. Strong down-modulation of surface BCR by repeated antigen stimulation contributes to lack of surface IgM and BCR signaling in normal B cells, explaining at least in part the anergic state of these cells [11, 28]. The same mechanism may be responsible for the indolent course observed for some CLL patients. Internalization of BCRs also results in a more immature N-glycosylation of surface-mannosylated IgM in normal B cells after prolonged antigen activation. This kind of immature N-glycosylation is also reported to be more prevalent in unmutated CLL [29]. This may suggest that the immaturely N-glycosylated surface IgM in part causes the more aggressive phenotype of unmutated CLL. Recent findings have revealed that BCR internalization is indeed employed in the regulation and fine tuning of the intracellular signaling cascades. Inhibition of BCR internalization was shown to result in dysregulated signaling that may reflect the state of some B cell malignancies [30].

Downstream Pathways

BCR signaling is able to switch between a proliferative, pro-survival, anti-apoptotic signaling and an anergic, apoptosis-promoting signaling. This seems to be reflected by the balancing between large biphasic calcium responses with activated NF-κB

vs. low calcium oscillation with activated ERK/NF-AT downstream in the BCR pathway [31]. These different downstream pathways convey in part the ability of normal B cells to switch between positive selection by foreign antigens and negative selection by self-antigens. A molecular signature of anergy in a subset of CLL patients (constitutively phosphorylated ERK, increased NF-AT transactivation) may be a correlate of negative selection by self-antigen and is associated with a more indolent course [32]. These downstream pathways of BCR represent ubiquitous pathways in normal cells as well as in the malignant counterparts. The ubiquitous usage of these pathways could represent a caveat in targeting them in CLL due to the expectation of multiple unwanted effects in other cell systems. Even so, the different pathways implicated in microenvironmental interaction that are so important for proliferation of CLL cells [8] converge with the BCR pathways on these kinases and transcription factors. Thus, these downstream pathways might very well point towards new targets for development of CLL treatment. Indeed, agents targeting NF-κB are in preclinical development with possible forthcoming testing in CLL.

Translation into Clinical Results

The elucidation of B cell pathways has rapidly been followed by development of new targeted therapies for CLL with impressive results. While these results are preliminary, they are promising for the treatment of CLL patients in forthcoming years. However, a caveat should be kept in mind: most of the targeting agents are actually multi-targeting drugs that inhibit several different kinases acting in different pathways. Thus, making inference from clinical effects to cellular mechanisms of action is difficult. Furthermore, the alluring concept of targeted therapies that achieve impressive results in initial clinical testing should still be rigorously studied in controlled clinical trials. It will be important to validate surrogate markers like progression-free survival (PFS) or complete/partial response (CR/PR) to overall survival [33, 34]. An additional consideration will be the quality of life with different treatment options. The current discussion of amendments to response criteria for CLL based upon the peripheral lymphocytosis seen with most of the evolving targeted therapies emphasizes the importance of reassessing response criteria [35]. At the same time, the benefits of targeted therapies should be implemented for patients as soon as possible. This is especially important, as current standards of treatment do not always meet the need for long-lasting control of CLL.

Targeting LYN

Upon activation of the BCR by antigen, LYN, an SRC family kinase, initiates BCR signaling by phosphorylating ITAMs on the cytoplasmic part of CD79A and CD79B that then recruit further components of the signaling pathway. LYN directly

phosphorylates SYK as part of the downstream pathways from the BCR complex. LYN also activates phosphatases that in turn inhibit signal transduction through the BCR [11]. By this double mode of action, LYN both activates and terminates BCR signaling. LYN-deficient mice have reduced numbers of B cells that at the same time are less responsive to acute BCR activation. These mice eventually develop a lupus-like autoimmune disease, thus pointing towards an essential function of LYN to both downregulate BCR activation and limit the expansion of autoreactive B cells [36, 37]. The balancing between activation and inhibition of the BCR pathway has been shown to depend on a close interaction between CD19 and LYN (see Fig. 12.1). Deficiency of CD19 represses the autoimmune phenotype of LYN-deficient mice [38]. Furthermore, mutations of CD79B in some lymphoid malignancies reduce LYN kinase activity, thereby promoting "chronic active BCR" signaling and constitutive NF-κB activation. These findings underscore the bidirectional function of LYN in B cells [20].

Dasatinib (BMS-354825)

Dasatinib is a dual SRC/ABL kinase inhibitor that is approved for use in CML [39]. In addition to targeting the BCR/ABL kinase in CML and several kinases from the SRC and TEC families (including both LYN and BTK involved in the BCR pathway [40], see Fig. 12.2a) it appears that dasatinib targets several other kinases in different kinase families [41]. In vitro, dasatinib induces variable degrees of apoptosis in CLL cells with no correlation between response and inhibition of LYN phosphorylation. However, the impact of dasatinib on the BCR pathway was demonstrated by in vitro apoptosis being inversely correlated with drug-induced inhibition of SYK phosphorylation. While dasatinib inhibited BCR signaling, stromal cell contact and CD40 stimulation antagonized the pro-apoptotic effect of the drug [42]. However, dasatinib has recently been shown to inhibit actin polymerization and migration in response to CXCL12 through inhibition of CXCR4 signaling [43]. This may be indicative of LYN inhibition, as mononuclear cells from LYN-deficient mice have shown impaired CXCR4-dependent migration [44].

Only one phase II study of dasatinib in relapsed/refractory CLL (15 patients enrolled) has been published to date [45]. The overall response rate (OR) for patients was 20 %. In addition, 4 patients (27 %) exhibited more than 50 % reduction in lymphadenopathy. Median PFS was 7.5 months. Myelosuppression was the primary toxicity, with grade 4 neutropenia and thrombocytopenia occurring in 40 % and 13 % of patients, respectively. As of November 2012, four active clinical studies with dasatinib in CLL (alone or in combination with fludarabine or fludarabine + rituximab) are registered at clinicaltrials.gov.

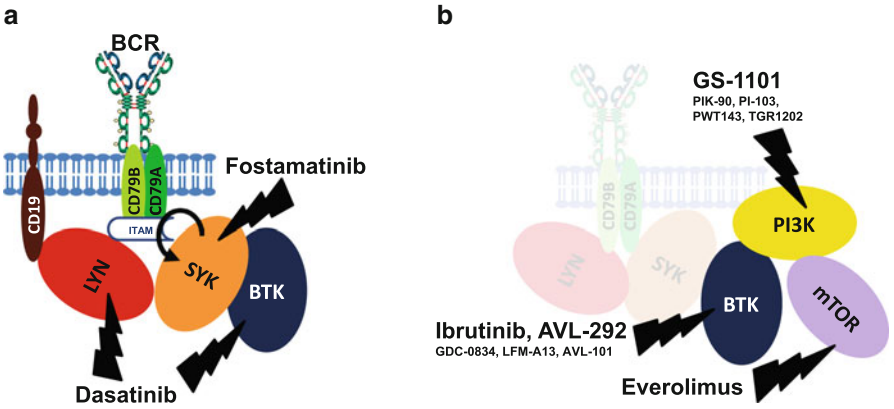

Fig. 12.2 (**a**) Inhibition early in the BCR pathway. LYN can be inhibited by dasatinib that was developed as a BCR/ABL inhibitor. BTK is also targeted by dasatinib. SYK is targeted by fostamatinib. See text and Table 12.1 for further details. (**b**) Inhibition in the intermediate part of the BCR pathway. PI3K can be inhibited by idelalisib and several other agents in preclinical development. Inhibition of BTK has been shown in clinical trials by ibrutinib and AVL-292 with other agents in preclinical development. Everolimus is an inhibitor of mTOR in clinical use for immunosuppression, being clinically tested for CLL. See text and Table 12.1 for further details

Targeting SYK (Spleen Tyrosine Kinase)

SYK is a non-receptor tyrosine kinase closely related to ZAP70 (essential for T cell receptor signaling) [46]. Mice with a genetic deletion of SYK have a severe impairment of B cell development at the pro-B cell to pre-B cell transition and lack mature B cells [47, 48]. Some redundancy between ZAP70 and SYK is indicated by ZAP70 expression during B cell development that can partially substitute for loss of SYK function. ZAP70 expression in CLL is correlated with unmutated phenotype and a more progressive disease course. Interestingly, the increased BCR response in ZAP70-expressing CLL cells is independent of its kinase activity. It appears to be mediated by inhibition of events that terminate the signaling response and/or interfere with internalization of the BCR, culminating in a more prolonged activation of SYK [10, 49].

Upon antigen binding to the BCR and through phosphorylation of ITAMs, LYN is recruited to the plasma membrane for subsequent phosphorylation of SYK. However, SYK is also directly recruited and activated by binding to two ITAMs of the BCR through its own two SH2 domains (see Mocsai et al. for a thorough review of SYK function [46]). This results in an amplification of the initial BCR signal and promotes the activation of downstream signaling cascades (see Fig. 12.1). In addition, SYK is involved in chemokine, integrin, and Fc-receptor signaling [46] and is thus an important factor in mediating multiple microenvironmental signals in CLL cells. Even though SYK is constitutively activated (pY352) in peripheral blood CLL cells, no correlations between

Table 12.1 Targeted therapies in development for CLL

Target	Function	Inhibitors	Response	PFS (median)	Toxicity	Trials
LYN	Activate and terminate BCR signaling	Dasatinib (+targets BTK)	3/15 PR + 6 nodal with lymphocytosis, phase II [45]	7.5 months	Neutropenia (40 % grade 4), thrombocytopenia (14 % grade 4), infection (14 % grade3)	Single agent, +fludarabine, +fludarabine/rituximab
SYK	Upstream amplification of BCR signaling, binds ITAM	Fostamatinib (and others)	6/11 PR, all lymphocytosis initially, phase I/II [57]		Diarrhea (54 % grade 1/2), neutropenia (25 % ≥ grade 3), thrombocytopenia (25 % grade 1/2), febrile neutropenia (8 % ≥ grade 3)	None in CLL, one in DLBCL
PI3Kδ	Intermediary in BCR pathway, also in tonic survival signaling for resting B cells	idelalisib	*n* 54, 26 % PR, 80 %: ≥50 % reduced lymph nodes, ≥50 % lymphocytosis in 58 %, phase I [72]	15 months	Pneumonia (24 % ≥ grade 3), neutropenia (24 % ≥ grade 3), thrombocytopenia (7 % ≥ grade 3), febrile neutropenia (7 % ≥ grade 3)	+Bendamustine, +/− rituximab (preliminary results [73]), +ofatumumab (preliminary results [74]), +fludarabine, +chlorambucil
BTK	Downstream of LYN/SYK in BCR signaling, also implicated in microenvironment interaction	Ibrutinib	11/16 PR (2 CR), all lymphocytosis initially, phase I [96]	26 months	Neutropenia (12.5 % ≥ grade 3), thrombocytopenia (7.2 % ≥ grade 3), respiratory (7.1 % ≥ grade 3), diarrhea (46.5 %)	+Rituximab (preliminary results [98]), +bendamustine/rituximab (preliminary results [99]), +ofatumumab (preliminary results [100]), +fludarabine/cyclophosphamide
		AVL-292	75/116 ≥ PR, +22 PR with lymphocytosis, phase Ib/II [97]		Diarrhea 54 %, all grades; respiratory 29 %, all grades	

(continued)

Table 12.1 (continued)

Target	Function	Inhibitors	Response	PFS (median)	Toxicity	Trials
mTOR	Downstream of PI3K in BCR pathway, immunomodulatory	Everolimus	4/22 PR + 8 nodal with lymphocytosis [112], minor response, lymphocytosis, phase I/II [110, 111]		Neutropenia (32 % ≥ grade 3), thrombocytopenia (50 % ≥ grade 3), infections (23 % ≥ grade 3), ARDS (5 % grade 4, class effect)	+idelalisib, +alemtuzumab, +rituximab (maintenance), +panobinostat, +bortezomib, +lapitinib, +sorafenib
		Temsirolimus	Minimal effect, phase II [113]			
CXCR4/ CXCL12	Microenvironment	Nox-A12				Single agent, +lenalidomide
		Plerixafor	5/14 PR (phase I, +rituximab) [130]			
BCL2	Mitochondria-mediated anti-apoptotic	Navitoclax	9/29 PR, phase I [134]		Neutropenia (28 % ≥ grade 3), thrombocytopenia (28 % ≥ grade 3), respiratory (21 % ≥ grade 3)	+Rituximab, +/− maintenance (preliminary results [136])
		ABT-199	Preliminary, phase I [137]		Diarrhea (24 %), infections (18 %)	
		Oblimersen	Modest effect, phase III [140]			None
		Obatoclax	Modest effect, phase I [142]			None
Cereblon	Immunomodulatory, both B and T cells	Lenalidomide	21/45 ≥ PR, tumor flare, phase II [151]		Neutropenia (70 % ≥ grade 3), thrombocytopenia (45 % ≥ grade 3), infections (5 % ≥ grade 3), febrile neutropenia (15 % ≥ grade 3)	+Cyclophosphamide, +fludarabine, +ofatumumab, +rituximab, +flavopiridol (results [152]), plerixafor, azacitidine

See text for further details and agents in preclinical development

the degree of SYK activation and clinical or biological features of more aggressive disease have been shown [50]. One possible explanation for this could be that CLL cells in peripheral blood only partially reflect the activity of signaling pathways in the tissue microenvironment [10].

Fostamatinib (R788, Oral Pro-drug of R406, the Active Metabolite)

Fostamatinib is the orally available formulation of an ATP-competitive kinase inhibitor that inhibits a number of other kinases in addition to SYK [51] (see Fig. 12.2a). Initially, fostamatinib was developed with a focus on treating inflammatory diseases. Treatment of CLL cells with fostamatinib in vitro inhibited BCR and integrin signaling, antagonized the protective effect of stromal cells, reduced migration to chemokines and adhesion to stromal components, and induced a moderate degree of apoptosis [50, 52, 53]. Thus, SYK inhibition antagonizes both BCR-dependent and BCR-independent pathways in CLL cells and thereby abrogates stimulatory input from the microenvironment. The significance of SYK inhibition in treating B cell malignancies has been supported by studies in animal models. Fostamatinib prevents disease progression both in TCL1 transgenic mice (in which antigen-dependent selection appears to play a similar role as in human CLL) and in a non-Hodgkin lymphoma model that depends on cooperation between MYC and BCR-derived signals [54, 55]. In the TCL1 transgenic mouse, SYK inhibition induced a transient increase in circulating lymphocytes, reduced the proliferation of malignant B cells, and prolonged survival of the mice [54]. The on-target effect of fostamatinib in the BCR pathway has been demonstrated by downregulation of BCR pathway-specific targets in patients treated with fostamatinib as well as downregulation of NF-κB and MYC targets. However, no correlation between inhibition of BCR signaling and response to treatment could be shown [56].

The only published clinical trial of an SYK inhibitor used fostamatinib in a phase I/II study which included 11 patients with CLL, of whom 6 (55 %) achieved a PR [57]. The response rate in CLL was the highest, ahead of diffuse large B cell lymphoma (DLBCL) (22 %), mantle cell lymphoma (11 %), and follicular lymphoma (10 %). The dose limiting toxicity was a combination of diarrhea (17 % $\geq$ grade 3), neutropenia (33 % $\geq$ grade 3), and thrombocytopenia (17 % grade 1/2). In the phase II portion of the trial the most common adverse events were reversible cytopenias (anemia: 13 % $\geq$ grade 3, neutropenia: 25 % $\geq$ grade 3), fatigue (50 % grade 1/2), diarrhea (54 % grade 1/2), and hypertension (29 %, 4 % $\geq$ grade 3). There is one ongoing study testing fostamatinib in DLBCL. However, there are currently no active trials in CLL. Late stage clinical trials in rheumatoid arthritis [58] are ongoing. Preclinical data on two more specific SYK inhibitors (PRT318 and P505-15) have recently been published [59].

Targeting PI3K (Phosphatidylinositol 3 Kinase)

The PI3Kδ isoform primarily expressed in leukocytes together with the ubiquitously expressed PI3Kα isoform is essential for B cell development. Mice lacking PI3Kδ/α show a virtual absence of B1 B cells and marginal zone B cells but still have follicular B cells [60]. The significance of the PI3K pathway downstream of the BCR is emphasized by rescue from apoptosis of resting B cells that lack BCR by introducing constitutive active PI3K into these cells [61]. However, PI3Kα and PI3Kδ fulfill somehow redundant functions in tonic BCR signaling, as demonstrated by constitutively active PI3Kα also being sufficient to rescue B cells that have lost BCR expression [62]. The PI3K pathway furthermore mediates migration signals in B cells, thus pointing towards an important role in the interaction of CLL cells with the microenvironment [60, 63]. The p85 subunit of PI3K binds via SH2 domains to tyrosine kinases or adaptor molecules, while the p110 subunit phosphorylates phosphatidylinositol substrates, thus generating PIP3. However, the mechanisms of activation/inhibition seem to be more complex, as one of the SH2 domains is in part inhibitory. This explains the oncogenic effect of a deletion of this domain identified in a Hodgkin lymphoma cell line [64]. PIP3 is a pivotal scaffold, recruiting the components for the functional signaling complex downstream of the BCR, including BTK, PLCγ2, and AKT (see Fig. 12.1). While BTK can be tyrosine-phosphorylated in the absence of PI3Kδ, downstream effects such as calcium mobilization and cell proliferation are severely impaired in the absence of PI3K [65].

Idelalisib (GS-1101, CAL-101)

Idelalisib has been shown to inhibit the PI3Kδ isoform and induce apoptosis in CLL cells [66] (see Fig. 12.2b). Microenvironmental signals relayed through the BCR and alternative pathways, including interaction with CD40L, BAFF, TNFα, and fibronectin, are inhibited by idelalisib [67, 68], resulting in inactivation of AKT and ERK. Moreover, idelalisib inhibits secretion of cytokines and chemokines from CLL cells in a dose-dependent manner. In vivo, CLL patients treated with idelalisib showed a rapid decrease in CCL3 and CCL4, previously shown to be upregulated in CLL cells in a BCR-dependent manner [10, 68, 69]. T cell viability is not affected by idelalisib, although T cell secretion of some inflammatory and anti-apoptotic cytokines seems to be inhibited [67]. Thus, T cell modulation may play a role in the function of idelalisib in CLL as well.

Safety and activity of idelalisib in hematologic malignancies were evaluated in a phase I study. Fifty-four patients with CLL were enrolled. The OR by IWCLL criteria [70] was 26 % [71, 72]. However, 80 % of patients had a reduction in lymphadenopathy by $\geq$50 %. Many of these patients did not meet criteria for response by IWCLL criteria due to a transient increase in the absolute lymphocyte count. As discussed recently by Cheson et al., the peripheral lymphocytosis seen

with most targeted drugs in CLL may warrant amendment of the response criteria for CLL [35]. PFS was not reached at >11 months and responses were independent of classic risk factors including response in patients with 17p deletion. Grade ≥3 adverse events included pneumonia (24 %), neutropenia (24 %), thrombocytopenia (7 %), neutropenic fever (7 %), anemia (6 %), and increased liver enzymes (6 %). Studies in which idelalisib is combined with bendamustine and/or rituximab, fludarabine, ofatumumab, chlorambucil, and chlorambucil + rituximab maintenance are currently being performed. Preliminary results from some of these studies have reported OR and 1-year PFS rates between 74 and 88 % (+bendamustine and/or rituximab) [73]. The combination with ofatumumab reported preliminary results with an OR of 82 % [74]. No major safety concerns have hitherto been reported for any of the combinations. However, it should be noted that these are very preliminary data.

Other PI3K Targeting Agents

Several PI3K inhibitors are in preclinical and early clinical studies in hematologic malignancies. Specificity of different inhibitors for specific isoforms of PI3K seems to confer specific effects, i.e., the PI3Kα inhibitors PIK-90 and PI-103 were more effective than PI3Kδ or PI3Kβ/δ-specific inhibitors at inhibiting CLL cell migration to CXCL12 and in antagonizing stromal cell-mediated survival signals [75]. Rigosertib, another PI3Kα/β inhibitor currently explored for myelodysplastic syndrome, induced apoptosis in CLL cells cultured in contact with stromal cells [76]. Despite the attractive option of selectivity, selective inhibitors of PI3K isoforms may be bypassed due to different PI3K isoforms assuming redundant functions [77]. Other PI3Kδ-specific inhibitors are also in preclinical and early clinical trials in CLL, including PWT143 and TGR1202 (recently reported preclinical findings [78, 79]). SAR245408 is a pan-PI3K inhibitor that is well tolerated in patients with solid tumors [80, 81].

Targeting BTK (Bruton's Tyrosine Kinase)

BTK is a member of the TEC kinase family that also includes TEC (B cells/T cells/liver cells), IL2-inducible T cell kinase (ITK), and BMX/ETK (bone marrow, endothelia, epithelia). Loss of BTK causes X-linked agammaglobulinemia with the absence of mature peripheral B cells and low serum immunoglobulin levels [82, 83]. BTK is a non-receptor tyrosine kinase recruited early in the BCR signaling cascade and closely linked to SYK, PI3Kδ, PLCγ, calcium signaling, and NF-κB activation [84]. Upon activation of the BCR pathway, BTK attaches to the plasma membrane through its pleckstrin homology domain that binds to PIP3 [85]. BTK appears to be essential only in B cells and is required for

BCR-induced calcium release, cell proliferation, and activation of the NF-κB pathway [86, 87]. BTK is shown to regulate actin dynamics and antigen processing during BCR activation [27]. A downstream target of BTK is PKCβ, which in turn phosphorylates IKK, resulting in release and translocation of NF-κB transcription factors to the nucleus [88]. The on-target effect of BTK inhibition has been confirmed by downregulation of BCR signaling targets and NF-κB activity in tumor cells from both the peripheral blood and lymph nodes of CLL patients treated with ibrutinib [89]. It has also been shown that BTK is involved in B cell trafficking through the pathways of CXCR4/CXCR5 chemokine receptors [90]. Using in vitro and in vivo models, CLL homing to CXCL12 and CXCL13, as well as secretion of chemokines upregulated by BCR and/or NF-κB activation (CCL3 and CCL4), was shown to be decreased by ibrutinib [91]. In the murine TCL1 transgenic model of CLL it was shown that lack of BTK activity slowed the development of CLL [92]. The lymphocytosis seen in some patients upon inhibition of BTK may be explained in part by inhibition of pathways involved in B cell trafficking and adhesion to stroma.

Ibrutinib (PCI-32765)

Ibrutinib binds covalently to the cysteine Cys-481 of BTK and thereby irreversibly inactivates the kinase [86, 93]. In addition to blockage of BCR signaling, it is reported that integrin-mediated adhesion as well as signaling, adhesion, and migration to CXCL12, CXCL13, and CCL19 is inhibited in primary CLL cells [94]. Herman et al. showed that ibrutinib not only inhibits BCR signaling but also disrupts the protective effect of stromal cells, and inhibits CD40, BAFF, TLR, and cytokine signaling [95]. Furthermore, ibrutinib decreases adhesion to stromal elements such as fibronectin and VCAM1 [91]. Human activated B cell (ABC)-like DLBCL cell lines with constitutive active BCR signaling were selectively inhibited by ibrutinib as well as by knockdown of BTK [21]. Trials in mice and dogs showed inhibition of BCR signaling with no effect on T cell receptor signaling. Levels of circulating autoantibodies were reduced in a mouse model of autoimmune disease and objective clinical responses were described in dogs with spontaneous non-Hodgkin lymphoma [87].

In the first clinical trial reported with ibrutinib treatment, an OR of 60 % across different B cell malignancies was reported. Out of 16 patients with CLL, 11 were categorized as responders, including two CRs [96]. More recently, OR of 71 % for treatment-naïve patients, 67 % for relapsed or refractory patients, and 50 % for high risk patients has been reported [97]. If PR with lymphocytosis is included according to the proposed amendment to CLL response criteria [35], the response rates increase to 81 %, 87 %, and 79 %, respectively. The estimated PFS at 26 months was 75 % for the relapsed/refractory cohort and 96 % for treatment-naïve patients, demonstrating a remarkable duration of response with single-agent therapy. Preliminary results from combination therapy with ibrutinib and

rituximab in high risk patients showed 17 out of 20 evaluable patients achieving PR, with the last 3 patients achieving a nodal PR with persistent lymphocytosis. Adverse events are reported to be manageable [98]. Another study investigating a combination of ibrutinib with bendamustine and rituximab showed preliminary OR in the same range without major toxicity [99]. A third study with preliminary results on the combination of ibrutinib and ofatumumab confirms the efficacy of targeting CD20 and the BCR pathway: an approximate 100 % OR in heavily pretreated patients [100].

Other BTK Inhibitors

Another selective, orally available BTK inhibitor, AVL-292, has been tested in early clinical trials. Preliminary data showed stable disease in 8 of 8 CLL patients, with a median decrease in lymph node size of 28 % and initial augmented peripheral blood lymphocytosis in most patients [101]. Several other BTK inhibitors are in preclinical testing (GDC-0834, LFM-A13, AVL-101) [102], with no clinical studies registered at clinicaltrials.gov.

Targeting mTOR (Mammalian Target of Rapamycin)

mTOR is an ubiquitously expressed serine/threonine kinase. It is a downstream mediator of BCR signaling (through PI3K/AKT) as well as a cell cycle regulator at the transition from G1 to S phase [103, 104]. Rapamycin (sirolimus, used as an immunosuppressant in organ transplants, isolated from *Streptomyces hygroscopicus*) was initially identified as a fungicide and later as an antitumor substance [105]. mTOR was identified as the primary target of rapamycin through binding of rapamycin to FKBP-12 [106]. The cell cycle arrest at G1 phase brought about by rapamycin treatment is dependent upon inhibition of cyclin-dependent kinase 2 (CDK2). In addition, survivin, which is expressed in proliferation centers of CLL cells in vivo, is downregulated, and cyclin D3, cyclin E, and cyclin A levels are reduced due to rapamycin inhibition of mTOR [104]. Preclinical data show that rapamycin-treated cells lacking functional p53 go into apoptosis, whereas p53 wild type cells arrest in G1 phase but remain viable [107]. Apoptosis induction mediated through mTOR inhibition, which depends on a lack of p53 function (del(17p) and p53 mutations), thus points towards a treatment option for CLL patients with the most dismal prognosis. In addition to the direct effects in B cells, inhibition of mTOR may deplete the oligoclonal, activated T cells found in CLL. Furthermore, it may block angiogenesis and VEGF, which is reported to be upregulated in cycling CLL cells [108]. The importance of mTOR signaling in CLL has been demonstrated in a mouse model. CLL cells arising in the TCL1 transgenic mouse (TCL1 overexpression is a coactivator of AKT, through which mTOR is activated) were transplanted into syngeneic mice.

Inhibition of mTOR signaling in this model was shown to both prevent and delay CLL development. At the same time, downstream targets of mTOR were shown to be inactivated in the CLL cells [109].

Everolimus (RAD001)

The development of everolimus, which has improved stability and oral availability compared to rapamycin, pioneered the way for trials of mTOR inhibitors in CLL [110]. In the first clinical trial of everolimus [111] in CLL, 4 out of 8 CLL patients were reported to have a reduction in adenopathy despite no objective response according to IWCLL criteria [70]. The second trial [110] showed signs of severe toxicity from immunosuppression and infectious complications (50 % $\geq$ grade 3, including two fatalities). At the same time, a tumor flare syndrome was seen. The immunosuppressive effect of everolimus in CLL was substantiated by a third trial [112] that also reported severe infectious complications (23 % $\geq$ grade 3, including two fatalities). In addition to general immunosuppression, pneumonitis was revealed as a class effect of mTOR inhibitors [113]. However, 4 out of 22 patients in this study achieved a PR. Additionally, 8 patients achieved a median decrease in lymph node size of 75 % while demonstrating increased peripheral lymphocytosis. Therefore, the phenomenon of mobilization of CLL cells from bone marrow and secondary lymphoid tissue to peripheral blood, similar to that reported for other targeted therapies, was also shown with mTOR inhibitors. The effect of everolimus treatment on redistribution of CLL cells to the peripheral blood was interpreted by the authors as a proof of effect. The reported peripheral lymphocytosis suggests an effect in combination therapy despite modest single agent activity [112]. However, the use of everolimus in combination therapy for CLL will await further clinical trials. Future trials also have to address strategies to handle the immunosuppressive effect of everolimus in CLL. At least eight active studies (excluding those in allogeneic stem cell transplantation) of everolimus combined with idelalisib, alemtuzumab, rituximab (maintenance), panobinostat, bortezomib, lapitinib, and sorafenib are registered at clinicaltrials.gov.

Other Drugs Targeting mTOR

Temsirolimus (CCI-779) has been evaluated for treatment of CLL in one single agent phase II study [113]. There are currently no ongoing trials registered at clinicaltrials.gov for CLL. Minimal single-agent effect in CLL was reported from the study. Other mTOR inhibitors are in preclinical and clinical development for other malignancies and immunosuppression, but no trials are registered for CLL.

Targeting of Other Tyrosine Kinases in CLL

AXL is a receptor tyrosine kinase recently identified in a constitutively phosphorylated state in microvesicles from CLL cells [114]. Further investigation identified AXL in a complex with several molecules of the BCR pathway, including LYN, SYK, PI3K, and PLCγ2 [115]. Preclinical studies on more or less specific inhibition of AXL by BMS777607 and LDC2636 showed effects on viability, polarization, and migration of CLL cells. Homing of CLL cells was also abrogated by AXL inhibition in a mouse model [116]. BMS777607 is currently being tested in clinical trials for other solid tumors. R428 is another specific AXL inhibitor with only preclinical data. Bosutinib (SKI-606) is an SRC/ABL kinase inhibitor developed for CML that also inhibits AXL. Both R428 and bosutinib have been shown to induce apoptosis in CLL cells [115]. These preclinical data point towards AXL as a new target in CLL.

ROR1 (receptor tyrosine kinase-like orphan receptor family member) is another receptor tyrosine kinase expressed in CLL cells. It functions as a receptor in the noncanonical Wnt pathway [117, 118]. In addition to targeting ROR1 by immunotoxins [119] and monoclonal antibodies [120], which results in apoptosis, a high throughput screening approach has recently identified a small molecule inhibitor of ROR1 (KAN0438063). Preliminary preclinical data demonstrate a selective apoptotic effect in CLL cells compared to normal peripheral blood mononuclear cells [121]. Thus, a new pathway for targeting CLL is being revealed. More research addressing the significance of ROR1 in microenvironmental interaction and intracellular signaling is warranted to advance clinical development in CLL.

PKCβ (protein kinase C β) functions downstream of BTK in the BCR pathway relaying signals further downstream through IKK to activation of NF-κB transcription factors. PKCβ has been targeted in preclinical and clinical trials for different B cell malignancies by enzastaurin. However, the clinical results have been disappointing and no further clinical trials in CLL are expected [122]. Nevertheless, clinical and preclinical experiences from targeting PKCβ may prove helpful in further exploring the BCR pathway and guide combination therapy approaches [123]. In regard to the targeting of IKK, only preclinical results with no translation into clinical studies have been reported, despite the strong preclinical rationale for targeting IKK as part of the BCR pathway that conveys signals to NF-κB [124].

NF-κB is constitutively activated in many cancers, including B cell malignancies and CLL. This is in part due to NF-κB being a major downstream point in the BCR pathway conveying BCR activation to pro-survival, proliferation, and migration signaling [11, 125]. NF-κB is one of the final signaling mediators of the BCR (and several other) pathways downstream of IKKα and PKCβ. NF-κB upregulates the anti-apoptotic BCL-2 family members [88]. PBS-1086 was recently shown in vitro and in xenograft models, of multiple myeloma to inhibit NF-κB. Apoptosis was induced in the models and synergism with bortezomib cytotoxicity was demonstrated [125, 126]. Thus, preliminary preclinical data point towards an anti-apoptotic

effect of NF-κB inhibition in CLL. Other NF-κB inhibitors like BAY 11-7082 have not been advanced into clinical development despite promising preclinical results [127]. Still, modulation of this key effector downstream of the BCR may be entering clinical development within the near feature.

Other Approaches to Target Tumor–Microenvironment Interactions

The effects of the above-described targeted therapies on the redistribution of CLL cells from lymph nodes to peripheral blood underscore the importance of microenvironmental factors in CLL. The outlining of both chemokines secreted by CLL cells (CCL3, CCL4, CCL22, IL-8) and chemokine receptors expressed on CLL cells (CXCR3, CXCR4, CXCR5, CCR7) warrants further research into modulation of these axes [128]. Preclinical and early clinical studies are currently addressing these pathways in the cellular microenvironment of CLL cells. The interaction of CXCL12 (SDF1α) with CXCR4 and CXCR7 is targeted by several agents, including Nox-A12 (an oligonucleotide-based, designed inhibitor of CXCL12, in clinical development) and plerixafor (AMD3100, a CXCR4 antagonist currently registered for mobilization of stem cells). Currently, one study of plerixafor in combination with lenalidomide is registered for the indication of CLL [129]. Plerixafor has also shown clinical effect in combination with rituximab (5 out of 14 patients achieved PR) [130]. Further clinical and preclinical data are awaited for outlining whether modulation of this chemokine pathway is feasible and beneficial in combination therapy for CLL.

Apoptosis Inducing Drugs Targeting the BCL-2 Family

The BCL-2 family of proteins govern the entry to the mitochondrial apoptotic pathway. Both pro- and anti-apoptotic family members exist in a delicate balance for decision-making regarding whether the CLL cells (or other cancer cells/normal cells) will live or die [131]. The apoptotic signals can originate from several cellular events, including DNA damage, growth factor activation, oncogene activation, or directly through dysregulation of BCL-2 expression, e.g., by t(14;18) translocation in follicular lymphomas [132]. Due to the plentitude of pro-apoptotic factors in most malignant cells, the anti-apoptotic BCL-2 family members balancing these pro-apoptotic factors are often occupied to capacity. This is contrary to the situation in normal cells, where the anti-apoptotic factors outnumber the pro-apoptotic factors. Therefore, pro-apoptotic drugs may more easily cause apoptosis in malignant cells than in normal cells [131].

Navitoclax (ABT-263, an Orally Available Analog of ABT-737)

Navitoclax is a BH3 mimetic that binds and inhibits the anti-apoptotic BCL-2 family members BCL-2, BCL_{-x} (BCL2L1), and BCL_{-W} (BCL2L2). Thereby, the repression of the pro-apoptotic BAX and BAK BCL-2 family members is relieved, and subsequently apoptosis is induced [133]. The first phase I trial of navitoclax in relapsed or refractory CLL patients showed an OR of 31 % (9 patients with PR out of 29 patients) [134]. Furthermore, nodal disease was reduced in 21 of 29 patients, splenomegaly resolved in 5 of 13 patients, and peripheral blood lymphocytosis was reduced by at least 50 % in 19 of 21 patients. Thrombocytopenia (28 % $\geq$ grade 3) was the dose limiting toxicity. In addition, gastrointestinal adverse events (76 % grade 1–2), neutropenia (28 % $\geq$ grade 3), and one possibly related event of progressive multifocal leukoencephalopathy were reported. A possible biomarker for response to navitoclax treatment was identified in the study. A high BIM:MCL1 ratio was statistically significantly associated with response among the subset of patients tested. This may be explained by displacement of BIM from BCL-2 by navitoclax, thus releasing BIM to antagonize pro-survival signaling through MCL1 that is not directly inhibited by navitoclax [135]. Currently, no actively recruiting phase II or further studies are registered for navitoclax at clinicaltrials.gov. Preliminary results from an open label phase II study randomizing between rituximab, rituximab + navitoclax, and rituximab + navitoclax + navitoclax maintenance showed 70 % OR in the arm with navitoclax maintenance vs. 35 % for the rituximab-only arm [136]. Bone marrow suppression, gastrointestinal symptoms, and increased laboratory values for liver enzymes were the common adverse events in the navitoclax-treated arms.

Other BCL-2 Targeting Drugs

Another BCL-2 inhibitor (ABT-199) with higher specificity than navitoclax for BCL-2 is being tested in phase I trials [137, 138]. Preliminary results are reported with effect in B cell malignancies.

The first strategy to target the BCL-2 protein used an antisense oligonucleotide [139]. A phase III trial showed only modest clinical activity of oblimersen (G3139 antisense) in lymphoid malignancies, including CLL [140]. However, the ability of oblimersen to lower BCL-2 protein levels in vivo has been questioned [131].

Another BH3 mimetic with pro-apoptotic effects through inhibition of pro-survival BCL-2 family members is obatoclax (GX015-070). In addition to the direct pro-apoptotic effect through the BCL-2 pathway, an indirect effect through NF-κB pathway-dependent apoptotic mechanisms has also been reported [141]. A phase I study reported very modest single-agent effect in advanced CLL patients,

with significant but transient infusion-related neurological adverse events probably caused by on-target effects [142]. No active studies for obatoclax in CLL are currently registered at clinicaltrials.gov.

Lenalidomide

Lenalidomide was developed as a derivative of thalidomide with antiangiogenic, antitumorigenic, and immunomodulatory activities. Despite activity in CLL and other hematological malignancies, the significance of the different mechanisms of actions for lenalidomide in different settings has not been fully resolved [143]. A few years ago, cereblon (CRBN) was identified as the intracellular binding partner for thalidomide and lenalidomide responsible for the teratogenic effect [144]. The interaction of lenalidomide with cereblon and the significance for T cell modulation was recently shown [145]. Multiple effects of lenalidomide on T cell signaling through the T cell receptor as well as co-stimulatory pathways seem to be implicated in the effect of the drug in CLL [146]. Through modulation of actin dynamics, lenalidomide restores the immunological synapse between T cells and CLL cells [147]. The inhibition of proteasome activity by binding of cereblon to the 20S core proteasome subunit β type 4 [148] and the identification of interferon regulatory factor 4 (also downstream of NF-κB) as a downstream target of cereblon [149] add to the complexity. Through induction of cytokine secretion (especially IL2 and INFγ) from both CLL cells and T cells, tumor recognition by the adaptive immune system is enhanced. Additionally, antibody-dependent cytotoxicity by NK-cells is reported to be enhanced by lenalidomide in CLL patients. Preliminary data showed that the level of cereblon expression pretreatment in CLL cells was not related to lenalidomide response [150]. The evolving understanding of the mechanisms of action for lenalidomide will guide development of combination approaches with lenalidomide for CLL.

The first clinical study of lenalidomide in CLL patients was published in 2006 [151]. An OR of 47 % was achieved among patients with relapsed or refractory CLL. For 9 % of the patients CR was achieved. Tumor flare reaction and tumor lysis syndrome were concerns in this as well as in subsequent trials, with slow-dose escalation and prednisolone investigated to ameliorate the side effects [152]. Tumor flare reaction is described as an initial tender swelling of CLL nodes with overlying erythema, sometimes seen as peripheral lymphocytosis or verified by CT scans for internal nodes. The tumor flare reaction is interpreted as a result of lenalidomide-driven modulation of the immune microenvironment. Studies have indicated that tumor flare reaction may predict for better responses; however, no differences in PFS were shown [153, 154]. Expression of co-stimulatory molecules on CLL cells that are in part responsible for the tumor flare reaction induced by lenalidomide can be abrogated by inhibition of PI3Kδ [155].

Based upon reported enhancement of antibody-dependent cytotoxicity by lenalidomide, combinations with rituximab were examined. Results from these studies indicate a possible superiority of the combination approach despite concerns for CD20 downregulation by lenalidomide [152]. Several other combinations with conventional chemotherapy (cyclophosphamide, fludarabine), ofatumumab, and flavopiridol (alvocidib, HMR-1275) have been reported, with OR between 46 and 90 % [152]. Preliminary data from the combination of lenalidomide and ofatumumab in relapsed CLL patients, including patients with del(17p), showed an OR of 68 % with less tumor flare reaction than in trials of single agent lenalidomide [156]. Several studies are registered at clinicaltrials.gov testing maintenance after conventional therapy or after transplantation, combinations with plerixafor, azacitidine (demethylation), chimeric IL2/CD40 cells, and NK-cell cord blood transplantation.

Peripheral Blood Lymphocytosis Due to Targeted Therapy in CLL

Among CLL patients treated with targeted drugs for CLL, peripheral lymphocytosis that typically resolves over weeks to months is commonly reported. Discussions whether this warrants the response criteria for CLL to be amended are ongoing [35]. Furthermore, this phenomenon may reveal new approaches for combination therapy. Disruption of microenvironment signaling by tyrosine kinase inhibitors or immunomodulatory drugs may sensitize the malignant cells to chemotherapy or indicate synergistic effects from targeting of additional pathways [8, 157]. Preliminary results from the combination of idelalisib with bendamustine show reduced initial lymphocytosis compared to single agent idelalisib [73]. Further preclinical and clinical trials are needed to address these aspects of CLL combination therapy.

Experimental Findings Guiding Future Approaches to Combination Therapy

A recent in vitro study on the impact of kinase inhibitors on sphingolipid metabolism points towards new combination therapies to overcome some types of chemoresistance in CLL. Lipoprotein lipase has been identified as a prognostic factor in CLL. Inhibition of lipases by orlistat was shown to induce apoptosis in CLL cells at a much lower concentration than in healthy B-lymphocytes [158]. Inhibition of BTK and PI3K has now been shown to inhibit glucosylation of ceramides and the mRNA levels of UDP-glucose ceramide glucosyltransferase (UGCG) in primary CLL cells. Furthermore, inhibition of UGCG directly or by BTK or PI3K inhibitors was shown to increase the sensitivity of primary CLL cells to apoptosis

induced by inhibition of BCL2 by navitoclax [25]. Thus, a possible synergy between BTK/PI3K inhibition and BCL-2 targeting through interaction with sphingolipid metabolism has to be tested in animal models or clinical trials.

Preliminary results on migration and chemokine secretion from ibrutinib-treated high risk CLL patients point towards redundancy in the BCR pathway in vivo [159]. As previously published [91], plasma levels of CCL3/CCL4 and secretion of these chemokines by CLL cells from ibrutinib-treated patients were significantly reduced. However, even though ibrutinib abrogated cell survival after anti-IgM stimulation of the BCR in pretreatment samples, this was not the case in posttreatment samples. This is in part conflicting with recent data demonstrating downregulation of BCR target genes in patients treated with ibrutinib [89]. It raises the question whether part of the IgM-triggered pro-survival signaling can bypass BTK inhibition, thus suggesting that attacking different points in the BCR pathway could be beneficial in CLL.

Different pathways work in parallel downstream of the BCR and microenvironmental signaling. As emphasized above, targeting only one kinase in the pathways may result in resistance due to redundancy in the signaling cascades, upregulation of parallel pathways, or mutations in the kinase. In addition to combination approaches, these preclinical considerations provide a rationale for the development of dual activity tyrosine kinase inhibitors like SAR245509 that targets both PI3K and mTOR (as well as several other kinases) [160]. The price of more broadly targeting kinase inhibitors may be increased toxicity. This remains to be explored during clinical development.

Combinations of lenalidomide and PI3K inhibitors are supported by preclinical data. Upregulation of co-stimulatory molecules on CLL cells involved in the tumor flare reaction described during lenalidomide treatment was decreased upon PI3Kδ inhibition [155]. The exploration of mechanisms of action for lenalidomide in CLL has further revealed the importance of interaction between CD4+ T cells that secrete IFNγ and CLL cells in the microenvironment of lymph nodes [153]. These indices of cross talk between immunomodulatory pathways and the BCR pathway should be further addressed both at the molecular biology level and in clinical trials.

Conclusion

The extraordinarily positive early results from phase I/II trials of targeted therapies in CLL bring a hitherto unseen optimism among clinicians, patients, and researchers. The concomitant development of a multitude of promising targeted therapies in CLL poses special challenges for the collaboration between academic researchers and pharmaceutical companies: the different combination approaches should be rigorously addressed in randomized trials. Comparison should be made to the current gold standards in treatment of different subsets of CLL patients. At the same time, the benefits of new treatments should be transferred to patients as soon as possible.

To address these commitments, several issues have to be addressed. The slow clinical course for most CLL patients emphasizes the need for validating surrogate endpoints like PFS, OR, and negativity for minimal residual disease in addition to overall survival and quality of life. The validation of these surrogate endpoints should be tested by systematic approaches [34]. Moreover, the evolving targeted therapies question the current response criteria for CLL [35]. Also, examination of whether new treatment regimens up front would be better than watchful waiting in terms of quality of life and OS is warranted [161]. New strategies for the design of clinical trials may address some of these issues. Ongoing incorporation of new treatment options into a randomized trial has been evoked for AML [162]. While continuously including new treatment options, those that do not meet predefined efficacy end points are discarded. The goal for this study design is to "pick a winner" from among several promising treatment options. This kind of study design may prove helpful in bringing new treatment options to patients while maintaining sound scientific testing of new targeted therapies.

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