Targeting B-cell receptor signaling kinases in chronic lymphocytic leukemia: the promise of entospletinib

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Abstract: The B-cell receptor signaling pathway has emerged as an important therapeutic target in chronic lymphocytic leukemia and other B-cell malignancies. Novel agents have been developed targeting the signaling enzymes spleen tyrosine kinase (SYK), Bruton's tyrosine kinase, and phosphoinositide 3-kinase delta. This review discusses the rationale for targeting these enzymes, as well as the preclinical and clinical evidence supporting their role as therapeutic targets, with a particular focus on SYK inhibition with entospletinib.

Keywords: B-cell receptor signaling inhibitors, chronic lymphocytic leukemia, entospletinib, idelalisib, lymphoid malignancies, phosphoinositide 3-kinase delta, spleen tyrosine kinase inhibitors

Entospletinib overview

B-cell receptor (BCR) signaling regulates many cell-fate decisions in normal B cells [Casola *et al.* 2004] and has emerged as an invaluable therapeutic pathway in numerous B-cell malignancies. The recent Food and Drug Administration (FDA) approvals of ibrutinib and idelalisib highlight the critical role of different kinase targets within the BCR signaling pathway. These drugs have unique spectrums of clinical activity with ibrutinib showing the greatest impact in chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), Waldenström's macroglobulinemia (WM) or lymphoplasmacytic lymphoma [Byrd *et al.* 2013, 2014, 2015; Treon *et al.* 2015; Wang *et al.* 2013] and possibly the activated B-cell subtype of diffuse large B-cell lymphoma (DLBCL) [Mathews Griner *et al.* 2014]. In contrast, idelalisib has the greatest activity in CLL and follicular lymphoma (FL) [Furman *et al.* 2014; Gopal *et al.* 2014]. Although some of these distinctions may be attributable to the drugs themselves, other differences are likely due to the pathway-kinase target. While both drugs inhibit BCR signaling, spleen tyrosine kinase (SYK), Bruton's tyrosine kinase (BTK), and phosphoinositide 3-kinase delta (PI3Kδ) have a variety of functions independent of BCR signaling, including transmission of signals from tumor necrosis factor (TNF) superfamily receptors, integrins, and cytokines. Further, there is anecdotal evidence that these drugs may work in sequence, even after resistance has developed to the alternative therapy [Mato *et al.* 2015]. For these reasons, the clinical developments of additional novel agents targeting BCR signaling will likely benefit patients with B-cell lymphoproliferative disorders.

B-cell receptor signaling

The BCR pathway includes kinases, adaptor proteins, and transcription factors. These proteins cooperate to initiate and transmit signaling from the BCR to complex downstream effectors through a variety of protein modifications and transcriptional changes [Rolli *et al.* 2002]. SYK, BTK, and PI3Kδ are critical signaling enzymes within the BCR signaling cascade that have emerged as important targets of novel agents [Fowler and Davis, 2013].

LYN

Signaling is initiated at the BCR, also known as a surface immunoglobulin (Ig). Although this molecule lacks any intrinsic signaling capability, it recruits LYN kinase to phosphorylate cluster of

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differentiation (CD)79, which in turn initiates the signaling cascade [Ackermann *et al.* 2015]. LYN functions to initiate signaling, but also has a negative feedback role that dampens overall signaling [Nishizumi *et al.* 1998]. Mice lacking LYN kinase manifest immune hyperactivity, suggesting that the negative feedback role for this enzyme plays a vital role in pathway balance [Lamagna *et al.* 2014]. Clinical trials of the pan-Src family inhibitor dasatinib have shown disappointing results in B-cell malignancies [Amrein *et al.* 2011].

Spleen tyrosine kinase

The dual CD79 sites phosphorylated by LYN kinase become a singular docking site for SYK. After binding to CD79, SYK undergoes autophosphorylation, resulting in enzyme activation [Kipps, 2007]. Thereafter, SYK activates a multitude of effectors, including the kinases BTK and PI3K [Gobessi *et al.* 2009]. Mice lacking SYK fail to develop B cells, and laboratory models of B cells modified to eliminate SYK have profound deficiencies in BCR signaling [Ackermann *et al.* 2015]. Because of its central role in BCR signaling, the SYK inhibitor R406 was studied preclinically for its therapeutic potential in B-cell malignancies [Sharman *et al.* 2007; Gobessi *et al.* 2009], and a clinical trial of fostamatinib (the oral prodrug of R406) pioneered the field of BCR signaling inhibition [Friedberg *et al.* 2010].

Fostamatinib demonstrated modest activity in DLBCL, but the more clinically meaningful benefit was identified in CLL, where 6 of 11 patients experienced a partial response to therapy. This was also the first demonstration of the unique pattern of clinical responses in which lymph node reduction was simultaneously associated with significant lymphocytosis [Friedberg *et al.* 2010]. This has also occurred with therapeutic inhibition of BTK and PI3Kδ [Byrd *et al.* 2013; Furman *et al.* 2014]. It has been subsequently recognized that therapy with BCR-signaling inhibitors results in significant alterations in cytokine signaling and egress of malignant lymphocytes from protective niches in the marrow, nodes and spleen [Herman *et al.* 2014]. Over time, these lymphocytes passively die because of the lack of BCR signaling.

Bruton's tyrosine kinase

Another molecule downstream of SYK is BTK, a kinase that helps propagate downstream signaling from the BCR. The gene for BTK lies on the X chromosome, and loss of BTK results in Bruton's agammaglobulinemia, in which patients lack B cells and fail to synthesize antibodies [Conley *et al.* 2009]. Therapeutic inhibition of BTK with the covalent inhibitor ibrutinib has resulted in profound clinical activity in a variety of B-cell malignancies [Byrd *et al.* 2013, 2014, 2015; Treon *et al.* 2015; Wang *et al.* 2013]. Several other BTK inhibitors have been evaluated, including CC-292 and GDC-0834; however, development of these molecules has been discontinued. Other BTK inhibitors, such as ONO-4059 (aka ONO/GS-4059) [ClinicalTrials.gov identifier: NCT01659255] and ACP-196 [ClinicalTrials.gov identifier: NCT02029443], are still in active development and may become available for the treatment of patients with B-cell malignancies.

In CLL, ibrutinib initially demonstrated substantial clinical activity in single-arm, phase II studies in both relapsed and refractory CLL, as well as in patients who were treatment naïve [Byrd *et al.* 2013; O'Brien *et al.* 2014]. Subsequent, randomized, phase III studies demonstrated the superiority of ibrutinib compared with ofatumumab in previously treated patients and compared with chlorambucil in treatment-naïve patients, as well as an additional benefit when added to bendamustine and rituximab (BR) [Byrd *et al.* 2014; Burger *et al.* 2015; Chanan-Khan *et al.* 2015]. Additional treatment indications include both MCL and WM based upon single-arm, phase II studies [Treon *et al.* 2015; Wang *et al.* 2013], and randomized studies are ongoing.

Phosphoinositide 3-kinase

PI3K propagates signals further downstream in the BCR pathway. PI3K activates the AKT and mechanistic target of rapamycin (mTOR) pathways, but also integrates signals from a variety of non-BCR cell-surface receptors [Puri and Gold, 2012]. The PI3K family has several isoforms, and the delta isoform is relatively specific to lymphocytes [Foster *et al.* 2012; Okkenhaug and Vanhaesebroeck, 2003]. Idelalisib was the first-in-class PI3Kδ inhibitor to achieve FDA approval in both FL and CLL.

Following promising activity in a phase I study [Flinn *et al.* 2014], idelalisib was evaluated in a variety of indolent non-Hodgkin's lymphomas (iNHLs) and demonstrated significant activity in marginal zone lymphoma (MZL), WM, small

lymphocytic lymphoma (SLL), and FL [Gopal *et al.* 2014]. Based upon a single-arm, phase II study of patients with disease refractory to both rituximab and alkylating agents [Gopal *et al.* 2014], idelalisib was granted FDA approval in both FL and SLL. Although idelalisib demonstrated an approximately 50% response rate in these refractory FL patients [Gopal *et al.* 2014], ibrutinib only led to responses in 11% of patients considered rituximab refractory [Bartlett *et al.* 2014], highlighting some of the important differences in the biologic spectrum of activity of the respective kinases.

In CLL, idelalisib was evaluated in two randomized studies in which patients were assigned to a CD20 antibody *versus* the same CD20 antibody in combination with idelalisib. In the rituximab *versus* rituximab and idelalisib study, marked improvements of the combination in overall response rate, progression-free survival (PFS) and overall survival (OS) led to FDA approval in this indication [Furman *et al.* 2014]. The similarly designed study with ofatumumab was recently reported with substantial improvements in outcome for patients receiving combinations containing idelalisib [Jones *et al.* 2015a]. A study of BR plus idelalisib in the frontline setting has been conducted, and the results are pending [ClinicalTrials.gov identifier: NCT01980888]. A study in the relapsed setting found that the addition of idelalisib to BR was superior to BR alone in reducing the risk of progressive disease and death, as well as increasing PFS and OS, with a safety profile consistent with previously reported studies [Zelenetz *et al.* 2015].

Rationale for further exploration of spleen tyrosine kinase inhibitors

Although clinical validation of BCR signaling inhibition was first demonstrated with therapeutic inhibition of SYK, clinical development of therapeutics against this target stalled for several years while the development of fostamatinib focused on rheumatologic indications. In the meantime, both ibrutinib and idelalisib showed striking improvements in clinical response rates and OS compared with existing standard therapies in a variety of treatment settings. Despite the clinically meaningful improvement in outcomes for patients receiving ibrutinib and idelalisib, emergence of resistant disease remains problematic [Maddocks *et al.* 2015]. There is a clinical need for additional agents targeting this pathway

that can be used to salvage patients with resistant disease or when simultaneously used in novel combinations to improve the efficacy of the existing agents. SYK remains an important, yet incompletely studied, target for additional drug development. Several agents, including fostamatinib, cerdulatinib, TAK-659, and entospletinib are currently being evaluated in this setting. Among SYK inhibitors in clinical development, entospletinib (also known as GS-9973) has reported the most comprehensive clinical experience to date. The following discussion will focus on the preclinical and clinical experience to date with this molecule.

Entospletinib

Entospletinib was designed following the initial clinical efficacy reports of the activity of fostamatinib in rheumatologic diseases and hematologic cancers [Weinblatt *et al.* 2008; Genovese *et al.* 2011; Friedberg *et al.* 2010]. Despite the demonstration of clinical efficacy, the development of fostamatinib was hampered by dose-limiting adverse effects (AEs), including a high incidence of hypertension, gastrointestinal effects, and neutropenia, which have been attributed to off-target kinase activities. To overcome this hurdle, entospletinib was synthesized and demonstrated substantially improved biochemical and cellular selectivity *versus* R406 [Currie *et al.* 2014; Burke *et al.* 2014]. Entospletinib modulates B-cell function by inhibiting BCR-stimulated, B-cell linker of NFκB (BLNK) phosphorylation, CD86 cell-surface activation-marker expression and proliferation, while showing enhanced cellular selectivity *versus* Janus kinase 2 (JAK2), cKIT, Fms-like tyrosine kinase 3 (FLT3), RET, and vascular endothelial growth factor receptor 2 (VEGFR2) *versus* R406 [Currie *et al.* 2014]. The improved selectivity increases the clinically achievable SYK target coverage [Ramanathan *et al.* 2013] without the concomitant dose-limiting AEs noted with fostamatinib, allowing for potentially improved efficacy and the ability to be safely combined with other targeted therapeutics in oncology.

Impact on B-cell receptor signaling

BCR signaling contributes to cell survival, proliferation and resistance to apoptosis in B-cell malignancies. Gene-expression profiling and immunoblotting in cellular assays of malignant B-cell lines and primary CLL samples demonstrate increased expression of BCR pathway
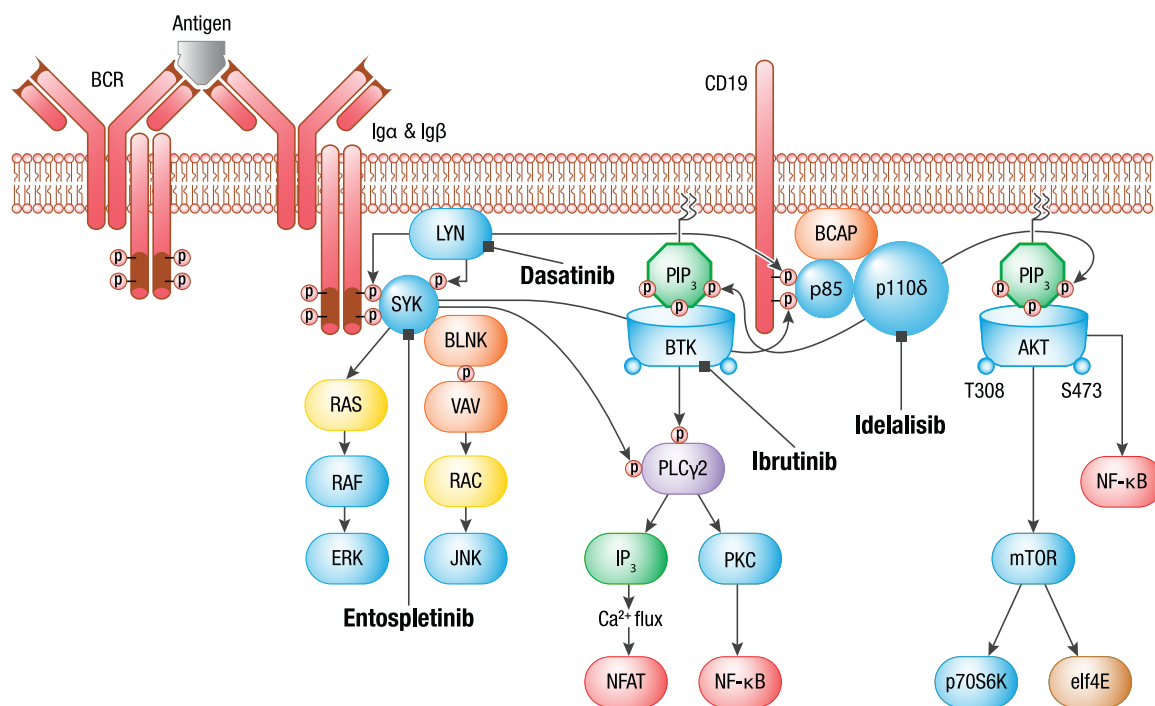
Figure 1. BCR signaling.
Adapted from Puri, K., Di Paolo, J. and Gold, M. (2013) B-Cell receptor signaling inhibitors for treatment of autoimmune inflammatory diseases and B-Cell malignancies. *Int Rev Immunol* 32: 397–427. Reprinted by permission of Taylor & Francis Ltd.

components and pathway activation, including phosphorylation of SYK, PLCγ2, signal transducers and activators of transcription 3 (STAT3) and extracellular signal regulated kinases 1 and 2 (ERK 1/2) compared with healthy B cells [Buchner *et al.* 2009]. As constitutive BCR signaling is required for B-cell survival, inhibition of the BCR-signaling cascade by SYK, BTK, and PI3Kδ inhibition validates this pathway as a pathogenic driver in B-cell malignancies (Figure 1).

Focusing on SYK and using a combination of mass spectroscopy and immunoblotting analysis, the phosphorylation of 13 signaling proteins were inhibited by entospletinib, and a subset of these phosphorylation sites were inhibited in basally activated, primary CLL cells [Di Paolo *et al.* 2014]. Inhibition of SYK by entospletinib in primary CLL cells resulted in cellular apoptosis [Burke *et al.* 2014; Jones *et al.* 2015b]. These observations confirm and extend earlier reports with structurally distinct SYK inhibitors (R406 and PRT06260) that showed reduction in AKT and ERK phosphorylation and induction of apoptosis in primary CLL cells [Gobessi *et al.* 2009; Buchner *et al.* 2009; Burke *et al.* 2014] and reduced PLCγ2 and AKT [Cheng *et al.* 2011] phosphorylation and proliferation of DLBCL cell

lines [Chen *et al.* 2008; Spurgeon *et al.* 2013]. Importantly, in CLL cases with the adverse Ig heavy chain unmutated BCR or ZAP70(+) prognostic markers, SYK inhibitors exerted stronger cytotoxicity than in mutated BCR samples from *in vitro* studies [Buchner *et al.* 2009]. The effect of SYK inhibition in CLL predicted by the *in vitro* studies was confirmed *in vivo* in the Eμ-TCL1 transgenic CLL mouse model, in which R406 inhibited BCR signaling, induced a transient increase in circulating lymphocytes, reduced the proliferation and survival of the malignant B cells and prolonged survival of the treated CLL mice [Suljagic *et al.* 2010]. Together, these observations demonstrate the importance of SYK function in multiple BCR cancer cell-signaling cascades.

In addition to directly affecting cellular signaling and apoptosis, BCR signaling facilitates attraction and retention of malignant B cells within the protective secondary lymphoid niches by altering the cytokine environment within these tissues [Quiroga *et al.* 2009; Buchner *et al.* 2009]. The negative CLL prognostic indicator, ZAP70 expression, results in enhanced BCR signaling and elevated expression of the chemokines CCL3 and CCL4 and chemokine receptors, including

CCR7, which are required for increased migration and homing to the protective lymph node microenvironment [Calpe *et al.* 2011]. SYK inhibition in CLL cells with entospletinib inhibited secretion of these cytokines, confirming the earlier results of R406 inhibition of CCL3 and CCL4 secretion [Burger *et al.* 2009; Hoellenriegel *et al.* 2012; Burke *et al.* 2014]. These results likely explain the CLL cell redistribution that results in rapid lymph-node shrinkage and lymphocytosis following inhibition with entospletinib and other SYK inhibitors in patients with CLL.

BCR signaling via SYK activity is known to affect other B-cell functions with poorly characterized roles in malignant B-cell survival, including regulation of B-cell activation markers necessary for T-cell activation [Currie *et al.* 2014], alteration of actin dynamics [Le Roux *et al.* 2007] and other cell-surface proteins.

Beyond the B-cell receptor: mechanism for future combinations

Independent of BCR signaling, SYK has been shown to be required for signaling downstream of Fc receptors, integrins and other receptors on immune cells [Mócsai *et al.* 2010; Tan *et al.* 2013; Currie *et al.* 2014]. As more is understood about SYK's role in integrating various signals, the potential for applying combination approaches to treat B-cell malignancies will undoubtedly emerge. Combinations with other BCR-signaling molecules to increase the depth of inhibition or combinations with distinct pathway inhibitors, such as BCL-2 inhibitors, could have the potential to improve and extend the durability of clinical responses.

CLL cells grow and proliferate in a microenvironmental tissue niche surrounded by mesenchymal stromal cells, nurse-like cells and lymphoma-associated macrophages in concert with T cells, natural killer cells and extracellular matrix components that interact and activate tumor B cells via BCR, TNF family members (i.e., BAFF, APRIL) and tissue-homing chemokine receptors and adhesion molecules [Herishanu *et al.* 2011]. This stromal interaction with CLL cells occurs independently of the BCR. In coculture experiments, entospletinib inhibited pSYK, pAKT, pMEK, pERK, pJNK2, pPKCδ and pS6 kinase in primary CLL cells incubated in the presence of HS-5 stromal cell coculture [Di Paolo *et al.* 2014], expanding the findings that R406 inhibited SYK

and AKT phosphorylations, as well as F-actin formation following chemokine and integrin stimulation in primary CLL cells [Buchner *et al.* 2010]. Stromal cell activation in the entospletinib studies resulted in a similar, although not identical, cellular phosphorylation profile that was inhibited by entospletinib treatment [Di Paolo *et al.* 2014], highlighting the modulation of BCR-independent signaling by entospletinib. Given the strong stromal cell effects on CLL survival, these results open the possibility of combining entospletinib with other pathway inhibitors to more completely suppress the pathologic signaling emanating from the stromal cell coculture.

Clinical trial combinations of entospletinib with idelalisib were initiated partially on the preclinical observation that the combination of idelalisib and entospletinib could more completely inhibit pAKT signaling in primary CLL cells with HS-5 stimulation [Burke *et al.* 2014]. Further justification for the combination was based upon the relatively distinct patterns of clinical responses in which idelalisib showed a more profound effect in iNHL [Gopal *et al.* 2014], whereas entospletinib showed a more substantial clinical benefit in CLL [Sharman *et al.* 2015a]. The results of the clinical study will be described later in the review.

Alternatively, combinations with other BCR inhibitors can be used to circumvent the development of resistance to a single inhibitor. For instance, resistance to ibrutinib has been characterized and arises from BTK-active site mutations and PLCγ2 activating mutations and other signaling-pathway activations, such as CARD11 [Woyach *et al.* 2014; Liu *et al.* 2015; Improgo *et al.* 2013; Chang *et al.* 2015]. *In vitro* experiments showed that the addition of entospletinib to ibrutinib-resistant cell lines harboring BTK active site mutations or PLCγ2 mutations insensitive to ibrutinib were sensitive to entospletinib, demonstrating the potential for an increased duration of response (DOR) with the dual combination of the two inhibitors [Liu *et al.* 2015; Chang *et al.* 2015]. Clinical studies looking at entospletinib treatment following ibrutinib or idelalisib failures are in progress [Clinicaltrials.gov identifier: NCT01799889; Sharman *et al.* 2015d].

SYK inhibition has been shown to alter actin dynamics in response to both BCR [Le Roux *et al.* 2007] and non-BCR-mediated (integrin) signaling [Pearce *et al.* 2011]. B-cell-microtubule assembly is also regulated in part by SYK [Faruki

et al. 2000], and pharmacologic SYK inhibition resulted in the block of the G1/S phase transition, resulting in cell-cycle arrest in DLBCL lines by preventing SYK-dependent activation of PLCγ2 and AKT [Cheng *et al.* 2011]. More recently, evidence that SYK inhibition may synergize with existing antimicrotubule cytotoxic agents [Yu *et al.* 2015] in epithelial tissues was reported. These reports highlight the potential for interplay between SYK and other microtubule agents with activity in hematologic neoplasms. Importantly, entospletinib synergized with the microtubule inhibitor, vincristine, and induced cytotoxicity via apoptosis in a wide range of malignant hematologic cell lines and demonstrated *in vivo* efficacy in a DLBCL cell xenograft [Axelrod *et al.* 2015].

SYK activation has been implicated as a mechanism for chemotherapy-induced drug resistance by upregulating antiapoptotic factors, such as MCL-1 in CLL cells, and inhibition of SYK with R406 in combination with fludarabine-abrogated stroma-mediated drug resistance by preventing upregulation of MCL-1 [Buchner *et al.* 2010]. Additional reports demonstrate that SYK inhibition can stabilize mRNA for prosurvival signaling proteins, such as BCL-XL [Wang *et al.* 2014]. These observations lead to the attractive hypothesis that SYK combinations with chemotherapeutics or prosurvival protein inhibitors, such as BCL-2 inhibitors, could reduce the incidence of chemotherapy-induced resistance and enhance the efficacy of prosurvival protein inhibitors, respectively. In a panel of primary CLL samples, the addition of entospletinib to a BCL-2 inhibitor, ABT-199, increased the level of apoptosis achieved in both the presence and absence of stromal-cell coculture, suggesting that an enhanced activity of this combination could have clinical benefit [Jones *et al.* 2015b; Bojarczuk *et al.* 2015].

Clinical experience with entospletinib

The safety and efficacy of entospletinib was evaluated in an open-label, phase II trial that enrolled five separate cohorts of patients with relapsed or refractory CLL, FL, other iNHLs (WM, SLL, MZL), MCL or DLBCL [Sharman *et al.* 2015a]. The safety analysis included all treated subjects across the five cohorts ($n = 186$). The most common nonhematologic AEs (>20%) of any grade included fatigue, nausea, diarrhea, decreased appetite, constipation, cough and headache. Grade 3 nonhematologic AEs (>2%) included fatigue, nausea and dyspnea. Treatment-emergent

serious AEs occurring in 2% or more of patients included dyspnea, pneumonia, febrile neutropenia, dehydration, and pyrexia. Treatment-emergent AEs occurring in 2% or more of patients and leading to study-drug discontinuation included fatigue, increased alanine transaminase (ALT), and headache. Grade 3 or more laboratory abnormalities occurring in greater than 10% of patients included neutropenia and increased ALT/aspartate transaminase (AST) [Sharman *et al.* 2015a].

Efficacy data have been reported for the patients with CLL ($n = 41$) [Sharman *et al.* 2015a]. Of 39 evaluable patients who had at least one postbaseline assessment, 94.9% achieved a reduction in adenopathy; a 50% or more decrease in the sum of products of the diameters (SPD) from baseline was achieved by 61.5% (95% CI, 44.6–76.6%) of subjects, and 33% had less than a 50% decrease in SPD from baseline. A total of 25 of 41 patients achieved a partial response (PR), whereas none achieved a complete response (CR). Three of the patients (7.3%) achieved a nodal response (>50% reduction in SPD) with persistent lymphocytosis. Of the 25 responding patients in the CLL cohort, the median DOR had not yet been reached (95% CI: 6.5 months to not reached) [Sharman *et al.* 2015a]. PFS at 24 weeks in the CLL cohort was 70.1% (95% CI: 51.3–82.7%). Median PFS was 13.8 months (95% CI: 7.7 months to not reached), based upon a median follow up of 7.7 months. Early results in a small cohort of entospletinib-treated patients either intolerant of, or resistant to, ibrutinib or idelalisib show reductions in lymph node volume in the majority of evaluated patients with low rates of early progression [Sharman *et al.* 2015d]. Longer follow up and a larger sample size of patients will be required before this strategy can be thoroughly evaluated.

Data in the FL cohort ($n = 41$) have also been reported [Sharman *et al.* 2015c]. A total of 37 of the 41 patients were evaluable for efficacy. Seven of 37 patients (19%) had a 50% or more decrease in SPD from baseline, and 19 of 37 patients (51%) had less than a 50% decrease in SPD from baseline. Seven of 41 patients (17%) achieved a PR, and one of 41 patients (2%) achieved a CR for an overall response rate of 20% (95% CI: 9–35%). Of the remaining patients, 20 (49%) had stable disease, 11 (27%) had progressive disease, and 2 discontinued the study prior to the first scheduled tumor assessment (one due to adverse events, and one withdrew consent). The 24-week

PFS in the FL cohort was 52% (95% CI: 33–67%), and the median PFS of 5.7 months was demonstrated with a median follow up of 3.6 months. Of the eight responding patients in the FL cohort, one patient was ongoing with a DOR of 11 months; three patients discontinued the study due to progressive disease and had DOR of 2.2, 4.5 and 10.9 months at the time of discontinuation; and four patients had no follow-up tumor assessment after responding (two were ongoing, and two discontinued the study due to AEs and were lost to follow up).

Several other indolent lymphoma studies have reported results. In a group of 15 patients with SLL, all patients evaluated experienced a reduction of adenopathy with an overall response rate of 67% and similar PFS to CLL patients [Sharman et al. 2015b]. Patients with lymphoplasmacytic lymphoma (n = 12) and MZL (n = 17) experienced response rates of 17% and 12% with 24-week PFS rates of 49% and 46%, respectively [Sharman et al. 2015c]. Clinical activity in DLBCL has not been reported. A summary of the clinical trials evaluating entospletinib monotherapy or combination therapy can be found in Table 1.

In separate studies, cerdulatinib demonstrated anecdotal responses, but because of the dose-finding nature of the study, evaluation of response rates in different disease states has not been reported [Hamlin et al. 2015]. A preliminary report of the activity of TAK-659 showed responses in three of seven evaluable patients with DLBCL (all PR) and in two of two evaluable patients with FL (one PR and one CR) [Petrich et al. 2015].

Combination clinical studies

Eradication of disease and prevention of relapse remain a challenge in CLL. The success of BCR-targeted therapies with BTK, PI3Kδ and SYK demonstrate significant efficacy but few cases of disease eradication. Combinations of these agents, as well as others, have been proposed and initiated based on the rationale of providing enhanced clinical benefit in the diverse genetic background of CLL, targeting pathways emanating from microenvironmental signals or to increase the DOR by overcoming potential resistance to single-target inhibition.

A pioneering study focused on two BCR-targeted inhibitors, idelalisib and entospletinib, that were clinically investigated based on the laboratory observation that these synergistically induced apoptosis in CLL and further disrupted chemokine signaling from clinical samples [Burke et al. 2014]. A rapid, robust median objective response rate of 60% at 10 weeks was observed with the combination in CLL. Despite the encouraging efficacy, this study was terminated early due to pneumonitis in 18% of patients, with two fatalities that were directly attributed to treatment-related pneumonitis [Barr et al. 2015]. Pharmacodynamic biomarkers in this study indicated increases of interferon γ, and interleukins 6, 7 and 8 occurred over time in patients who ultimately developed pneumonitis, without similar trends observed in other patients. Importantly, this study highlights the need for clinical caution when evaluating novel combinations of targeted agents.

Based upon laboratory evidence of SYK regulation of microtubule dynamics, treatment of B-cell lineage acute lymphoid leukemia (B-ALL) with the combination of entospletinib and vincristine is currently being clinically evaluated [ClinicalTrials.gov identifier: NCT02404220; Minden et al. 2015]. Additional phase I studies for patients with iNHL or aggressive NHL are evaluating entospletinib in combination with vincristine-containing regimens, such as R-CHOP, dose-adjusted R-EPOCH, and R-CVP [ClinicalTrials.gov identifier: NCT02568683]. Other pathways for which combination strategies with entospletinib may be therapeutically beneficial include the use of BCL-2 inhibitors or other BCR signal proteins, such as BTK. Many novel combinations have been supported by preclinical studies or have reached clinical development (Table 2). This trend for chemotherapy-free, combination therapeutic evaluations will continue as efforts to eradicate hematologic malignancies are pursued. As initial clinical results are reported for the selected combinations, the safety and tolerability of given combinations will be informative for learning how to best combine these novel agents to achieve clinical effect in the future.

Conclusion

Targeted inhibition of BCR signaling has become a validated strategy across a variety of lymphoid malignancies. The FDA approvals of ibrutinib and idelalisib highlight the exciting potential of drugs targeting this pathway, yet also expose the need for more understanding of how BCR signaling proteins interact with one

Table 1. Clinical trials evaluating entospletinib as monotherapy or in combination therapy.

Entospletinib: monotherapy or combination therapy	Condition	Title	Reference/ ClinicalTrials.gov identifier
Monotherapy	Relapsed or refractory CLL	An open-label phase II trial of entospletinib (GS-9973), a selective SYK inhibitor, in CLL	Sharman *et al.* [2015a] NCT01799889
Monotherapy	CLL refractory to prior therapy with ibrutinib or idelalisib	Clinical activity of entospletinib (GS-9973), a selective SYK inhibitor, in patients with CLL previously treated with an inhibitor of BCR pathway signaling	Sharman *et al.* [2015d] NCT01799889
Monotherapy	Relapsed or refractory iNHL (FL, LPL/WM, SLL, MZL)	Phase II trial of entospletinib (GS-9973), a selective SYK inhibitor, in CLL and SLL	Sharman *et al.* [2015b] NCT01799889
		Phase II trial of entospletinib (GS-9973), a selective SYK inhibitor, in iNHL	Sharman *et al.* [2015c]
Combination therapy with idelalisib	Relapsed or refractory CLL	Phase II study of idelalisib and entospletinib; pneumonitis limits combination therapy in relapsed refractory CLL and NHL	Study halted due to excessive immune-related toxicities Barr *et al.* [2015] NCT01796470
Combination therapy with vincristine and dexamethasone	Relapsed or refractory ALL	Safety and efficacy of entospletinib with vincristine and dexamethasone in adults with relapsed or refractory ALL	Minden *et al.* [2015] NCT02404220
Combination with vincristine and vincristine-based combination chemotherapy	Relapsed or refractory NHL	Safety and efficacy of entospletinib (GS-9973) combined with VCR and VCR-based combination chemotherapy in adult participants with NHL	NCT02568683

ALL, acute lymphoid leukemia; BCR, B-cell receptor; CLL, chronic lymphocytic leukemia; FL, follicular lymphoma; LPL, lymphoplasmacytic lymphoma; MZL, marginal zone lymphoma; iNHL, indolent non-Hodgkin's lymphoma; NHL, non-Hodgkin's lymphoma; SLL, small lymphocytic lymphoma; SYK, spleen tyrosine kinase; VCR, vincristine; WM, Waldenström's macroglobulinemia.

another and mediate crosstalk with other pathways regulating cytokine signaling, microtubule dynamics, microenvironment signaling and cell-survival proteins. As a key molecule in BCR signaling, SYK regulates many critical functions, yet the clinical development of SYK inhibitors has lagged behind the development of BTK and PI3Kδ inhibitors. Although the single-agent activity of entospletinib in CLL is impressive, regulatory-approval strategies for the molecule are considerably more difficult now than they might have been only several years ago. Whether entospletinib can find a route to approval as a single agent for patients either resistant to or intolerant of approved novel agents remains speculative. Alternatively, strategies to enhance activity or prevent resistance when given in combination with approved agents will likely be required. The efficacy and relative tolerability of entospletinib provides the potential for it to be a backbone for future therapeutic combinations.

In contrast, the activity in iNHL is likely insufficient to gain FDA approval as a single agent, yet emerging preclinical data and ongoing clinical

Table 2. Novel combination therapies in development.

Sponsor	Combination	Condition	Title	Study design	Reference/ ClinicalTrials. gov identifier
AbbVie	Duvelisib (PI3Kδ,γ) + ABT-199 (BCL2)	CLL	Elevated level of BCL-2 is the primary target for inhibition during duvelisib (IPI-145) therapy: ABT-199 neutralizes the resistance mechanism in CLL	Preclinical evaluation of PBMCs collected from CLL patients who participated in phase I study of duvelisib treatment	Patel *et al.* [2015]
AbbVie	Duvelisib (PI3Kδ,γ) + ibrutinib (BTK)	20 cell lines, including DLBCL, FL, T-cell and MCL, MM	High throughput, *in vitro* combination sensitivity screen in hematologic malignances with the PI3K-δ,γ inhibitor, duvelisib	Preclinical evaluation measuring growth inhibition in a 6 × 6 or 9 × 9 dose combination matrix	Faia *et al.* [2015]
AbbVie	ABT-199 (BCL2) + ibrutinib (BTK)	MCL	Multi-institution, phase I/Ib study of ibrutinib with ABT-199 in relapsed/refractory MCL	Phase I, nonrandomized, open-label, single group	NCT02419560
Acerta	ACP-196 (BTK) + ACP-319 (PI3K)	CLL	A multicenter, open-label, phase I pilot study of ACP 196 in combination with ACP-319 in subjects with relapsed/refractory CLL	Phase I, nonrandomized, open-label, single group	NCT02157324
Acerta	ACP-196 (BTK) + pembrolizumab (anti-PD-1)	NHL, MM, Hodgkin's lymphoma, CLL, Richter's syndrome, WM	A phase Ib/II proof-of-concept study of the combination of ACP-196 and pembrolizumab in subjects with B-cell malignancies	Phase I/II nonrandomized, open-label, single group	NCT02362035
Gilead	Lenalidomide (immunomodulator) + idelalisib (PI3Kδ)	Relapsed FL	A phase I trial of lenalidomide and idelalisib in recurrent FL	Phase I, open-label, single group	Study halted due to excessive immune-related toxicities NCT01644799
Gilead	Entospletinib (SYK) + vincristine (antineoplastic)	ALL	Phase Ib, open-label, dose escalation and expansion study evaluating the safety and efficacy of entospletinib (GS-9973) with vincristine and dexamethasone in adult subjects with relapsed or refractory ALL	Phase I, open-label, dose escalation expansion	NCT02404220
Gilead	Ibrutinib (BTK) + idelalisib (PI3Kδ)	B-cell malignancies	A phase Ib dose escalation and dose expansion study of ONO/GS-4059 combined with idelalisib in subjects with B-cell malignancies	Phase I, open-label, single group	NCT02457598
Gilead	Lenalidomide (immunomodulator) + idelalisib (PI3Kδ)	MCL	A phase I/randomized phase II trial of idelalisib and lenalidomide in patients with relapsed/refractory MCL	Phase II, randomized, open-label, parallel group	NCT01838434

(Continued)

Table 2. (Continued)

Sponsor	Combination	Condition	Title	Study design	Reference/ ClinicalTrials. gov identifier
Gilead	Entospletinib (SYK) + idelalisib (PI3Kδ)	CLL, MCL, DLBCL, iNHL	A phase II, open-label study evaluating the efficacy, safety, tolerability, and pharmacodynamics of GS-9973 in combination with idelalisib in subjects with relapsed or refractory hematologic malignancies	Phase II, open-label, single group	Manuscript in progress; study halted NCT01796470
TG Therapeutics	TGR-1202 (PI3Kδ) + TG-1101 (CD20)	CLL, BCL	Ublituximab, a novel glycoengineered anti-CD20 mAb, in combination with TGR-1202, a next generation once daily PI3Kδ inhibitor, demonstrates activity in heavily pre-treated and high-risk CLL and BCL	Phase I, nonrandomized, CLL and NHL cohorts	Lunning *et al.* [2014]
TG Therapeutics	TGR-1202 (PI3Kδ) + TG-1101 (CD20) + ibrutinib (BTK)	CLL, NHL	A phase I/Ib study evaluating the efficacy and safety of ublituximab, a third-generation anti-CD20 mAb, in combination with TGR-1202, a novel PI3Kδ inhibitor; and ibrutinib, in patients with B-cell malignancies	Phase I/Ib, nonrandomized, open-label, single group	Lunning *et al.* [2015] NCT02006485
TG Therapeutics	Ibrutinib (BTK) + ublituximab (CD20)	CLL, MCL	A multi-center phase II study with safety run-in evaluating the efficacy and safety of ublituximab in combination with ibrutinib in patients with select B-cell malignancies	Phase I/II, open-label, single group	Sharman *et al.* [2014] Full publication in progress NCT02013128
TG Therapeutics	Ibrutinib (BTK) + ublituximab (CD20)	High-risk relapsed or refractory CLL	A phase III, randomized study to assess the efficacy and safety of ublituximab in combination with ibrutinib compared to ibrutinib alone, in patients with previously treated high-risk CLL	Phase III, randomized, open-label, parallel group	NCT02301156

ALL, acute lymphoid leukemia; BCL, B-cell lymphoma; BTK, bruton's tyrosine kinase; CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; iNHL, indolent non-Hodgkin's lymphoma; mAb, monoclonal antibody; MCL, mantle cell lymphoma; MM, multiple myeloma; NHL, non-Hodgkin's lymphoma; PBMC, peripheral blood mononuclear cell; PI3K, phosphoinositide-3 kinase; SYK, spleen tyrosine kinase; WM, waldenström's macroglobulinemia.

trials focusing on the interaction of entospletinib with microtubule agents could open several regulatory strategies for the molecule in a variety of lymphoid malignancies. If SYK inhibition enhances the activity of agents such as vincristine, or the auristatins used in antibody drug conjugates, several routes to approval could emerge.

There is little question that SYK plays a central role in B-cell biology, and entospletinib has demonstrated intriguing potential as a therapy. Demonstrating that SYK inhibitors can play a central role in the treatment of B-cell malignancies remains an area of intense laboratory and clinical investigation.

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Conflict of interest statement
J. Sharman received research funding and honoraria from Gilead Sciences, Inc., Pharmacyclics Inc., and Genentech, Inc., and received research funding and consulting fees from Celgene Corporation.

J. Di Paolo is employed at Gilead Sciences, Inc., and owns stock in the company.

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