Review article

DOI: http://dx.doi.org/10.18320/JIMD/201502.0283

Journal of International Medicine and Dentistry 2015; 2 (2): 83-92

JOURNAL OF INTERNATIONAL MEDICINE AND DENTISTRY

To search.....to know.....to share

JIMD

ISSN.No.2350-045X

Targeting B-Cell Receptor Signaling – Changing the treatment landscape of B-Cell Lymphoma

Santhosh Sadashiv [1], John Lister [2]

Abstract:

Introduced in 1998, the anti-CD20 monoclonal antibody rituximab, with its unique mechanism of action, was the first agent to improve survival in patients with B-cell lymphoma (BCL) treated with chemotherapy. Laboratory investigation of the B-cell receptor signaling pathway identified the critical nature of this pathway for normal B-cell development, survival and proliferation. Further investigation showed that lymphoma cell lines were also dependent upon this pathway and hence small molecule inhibitors of critical proteins in the pathway were synthesized and shown to be cytotoxic. Subsequent translation to the clinic has shown impressive activity in some types of B-cell lymphoma. The aim of this article is to provide an overview of the constituents of the BCR signaling pathway, to illustrate how addiction to this pathway is critical for survival of some BCL, and to summarize the clinical experience with novel small molecule inhibitors of specific proteins in the BCR pathway. We speculate that combination of these agents with newer drugs, each with a unique mechanism of action might lead to improved therapy and the eventual elimination of standard chemotherapy from our therapeutic arsenal.

Keywords: B-cell lymphoma, B-cell receptor, Small molecule inhibitors

In the decade that began 1980, several phase 2 clinical trials suggested superiority of more intensive chemotherapy regimens over the standard CHOP (Cyclophosphamide/Adriamycin/Vincristine/Prednisone) regimen for the treatment of B-Cell Lymphoma (BCL)[1]. However, when tested in a randomized phase 3 controlled clinical trial, Fisher and colleagues demonstrated that CHOP provided equivalent outcome to the more intensive regimens with less toxicity[2]. The outcome with CHOP chemotherapy was improved with the addition of rituximab to the CHOP regimen[3]. This improved outcome without significantly increased toxicity changed the treatment landscape of BCL and signaled the beginning of targeted therapy in oncology[3]. Identification of CD20, a glycosylated phosphoprotein expressed on most B lymphocytes, and demonstration of its suitability as a target for monoclonal antibody therapy led to the development of rituximab. Rituximab, a chimeric monoclonal antibody directed against CD20 is believed to exert its cytotoxic effect by triggering apoptosis, mediating antibody dependent cellular cytotoxicity (ADCC) and initiating complement dependent cytotoxicity (CDC)[4]. Although, the addition of rituximab or other CD20 directed monoclonal antibodies to conventional combination chemotherapeutic agents has significantly improved progression free and overall survival [5], it is not uncommon for patients with BCL to develop toxicity related to cytotoxic chemotherapy. The appearance of resistance to therapy leading to relapse of lymphoma or transformation to aggressive type leaves patients with limited curative treatment options and frequently results in their demise[6].

Recent understanding of the B-Cell receptor and its signaling mechanism is rapidly changing the treatment landscape of BCL. The focus of this article is to

briefly discuss BCR signaling and present an overview of some of the novel drugs targeting BCR signaling pathways.

Molecular Biology of the BCR

B cell development requires successful rearrangement and expression of the immunoglobulin heavy (IgH) and light (IgL) chain genes which together form the BCR on the cell surface[7]. BCR engagement signals downstream pathways and promotes development, expansion and survival of normal B cells[8, 9] (**Figure I**). These same pathways are used by malignant B cells to drive proliferation, growth and survival[10]. Engagement and activation of the BCR occurs by diverse mediators and ranges from autologous stimulation from self-origin antigens, microbial derived antigens and constitutive signaling occuring as a result of somatic mutation of genes with immunoreceptor tyrosine-based activation motif (ITAM) in the signaling modules of CD79A and CD79B[9]. The activated BCR leads to a cascade of well described phosphorylation events involving multiple kinases such as the Src family kinases (SFK, Lyn), spleen tyrosine kinase (SYK), Bruton Tyrosine Kinase (BTK) and Phosphoinositide 3-kinase (PI3K). These proximal tyrosine kinase pathways in turn activate distal signaling pathways involving such molecules as NFκB, MAPK, AKT/mTOR that drive proliferation, growth and survival[11]. Understanding the components of these pathways as prospective targets for inhibition by small molecule inhibitors (SMI) has resulted in discovery of novel targeted agents.

Bruton Tyrosine Kinase (BTK) Pathway Inhibitors

BTK is a cytoplasmic tyrosine kinase essential for BCR signaling. BTK is expressed in all B cells from early precursor to mature forms (except plasma cells) and is essential for B cell survival. Following active BCR signaling, BTK is phosphorylated by SYK and in-turn leads to activation of downstream pathways such as protein kinase C beta, CARD11 and NF-kB[12]. Ibrutinib is an orally bioavailable selective kinase inhibitor that covalently binds to the Cys-481 residue of BTK[13]. In early clinical trials, Ibrutinib demonstrated clinical antitumor activity, near-complete target binding based on fluorescent studies with a mild to moderate toxicity profile. There was no reported cumulative hematological or non-hematological toxicity on prolonged dosing. Responses were seen in multiple histological variants of BCL. These results prompted phase 2 studies of Ibrutinib in patients with relapsed/refractory indolent and aggressive BCL[14, 15]. Given higher response rates in Mantle Cell Lymphoma (MCL) (ORR 68%) and Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL) (ORR 71%) when compared to other BCL subtypes, Ibrutinib was approved for the treatment of relapsed /refractory MCL and CLL/SLL[14, 15]. Ibrutinib has also received approval as front line therapy for patients with CLL who harbor the 17p deletion following demonstration of an ORR of 68 % in these patients. The presence of the 17p- makes cells resistant to cytotoxic chemotherapeutic agents[15]. When tested in relapsed diffuse large B cell lymphoma (DLBCL) higher responses were seen in the activated B-cell (ABC) subtype at 40% compared to the germinal center B cell (GCB) subtype at 5%[16]. This was as expected due to the frequent presence of a BCR activating mutation leading to constitutive BCR signaling seen in the ABC subtype of DLBCL but not in the GCB subtype[17]. Identification of the MYD88 mutation and its involvement in aberrant BCR signaling led to testing of ibrutinib in patients with relapsed refractory Waldenstrom's macroglobulinemia who frequently harbor the MYD88 mutation[18].

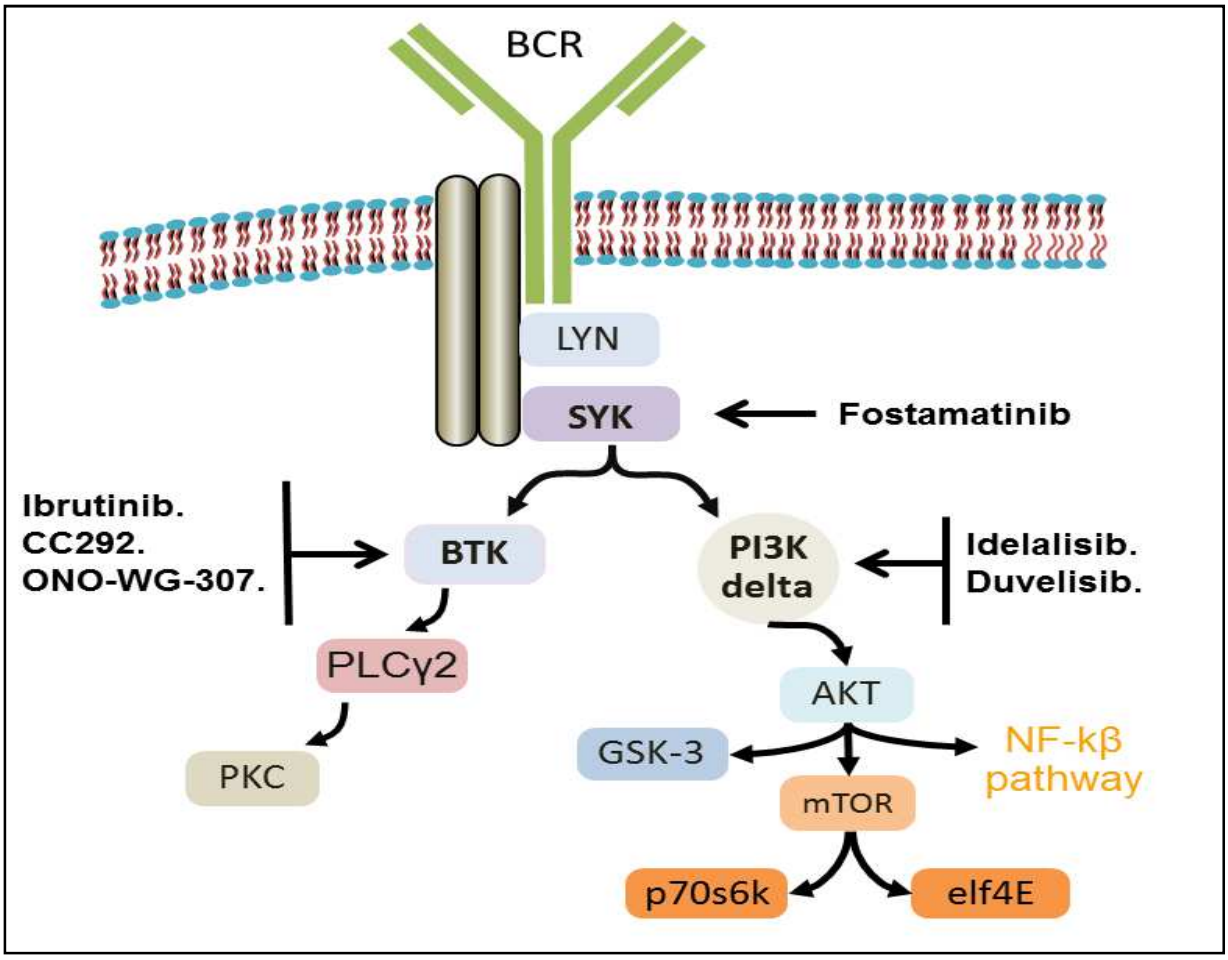

Figure I: BCR signaling pathway and site of inhibition by new targeting agents in B-cell NHL. *BCR, B-cell antigen receptor; BTK, Bruton's tyrosine kinase; GSK-3, glycogen synthase kinase 3; mTOR, mammalian target of rapamycin; NF-kβ, nuclear factor kappa-light-chain-enhancer of activated B cells; PI3K, phosphatidylinositide 3-kinases; PKC, protein kinase C; PLC, phospholipase C; Syk, spleen tyrosine kinase.*

(Adapted with modification from Davis et al. Nature 2010; 463: 88-92)

In a phase 2 multicenter study involving 63 patients with relapsed refractory Waldenstrom's Macroglobulinemia harboring the MYD88 mutation, treatment with ibrutinib resulted in rapid reduction in serum IgM levels and improvement in hematological parameters. These responses were durable with 87% of patients continuing on treatment at a median of 9 months[19].

With the demonstration of efficacy, durable response and a mild toxicity profile in the relapsed refractory setting, investigators have designed clinical trials with Ibrutinib either as a single agent or in combination with other therapeutic agents as frontline therapy for the treatment of BCL. (See **Table I**).

At the time of writing Ibrutinib is the only commercially available drug, but there are other BKT inhibitors in various stages of development. Examples include CC292 (Onyx Pharmaceuticals) and ONO-WG-307 (ONO Pharma UK), where early studies have demonstrated significant BTK

Table I: BTK Inhibitor combination studies

	Phase	N	Histology	ORR	Study
Ibrutinib+Rituximab	2	50	MCL	85%	Wang et al [45]
Ibrutinib + R- CHOP	3	TBD	Non GCB-DLBCL	Ongoing	NCT01855750
Ibrutinib+ R-CHOP	1b	33	Aggressive NHL	100%	Younes et al [43]
Ibrutinib+ Benadmustine+ Rituximab (BR)	1	11	NHL	38%	Blum et al.[39]
Ibrutinib + BR	1 / 2	30	CLL	90%	O'Brien et al.[36]
Ibrutinib + BR and Ibrutinib + R CHOP	3	TBD	iNHL	Ongoing	NCT01974440
Ibrutinib+ Rituximab	3	TBD	WM	Ongoing	NCT02165397

binding ability and activity against some BCL [20, 21].

Phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) Inhibitors

The PI3k /AKT pathway is well described and is part of proximal intracellular signal transduction pathways affecting many intracellular biological functions critical for metabolism, growth and proliferation [22]. Although existence of multiple isoform has been described, p110 delta and p110 gamma isoforms are predominantly expressed in cells of hematopoietic origin and hence represent a potential target for therapy in hematological malignancy. Aberrant activation of this pathway has been implicated in initiation and maintenance of tumors[23]. Inhibition of this pathway has demonstrated antitumor activity via suppression of cell signaling and angiogenesis[24, 25].

Currently, Idelalisib an orally administered selective inhibitor of PI3K delta isoform, is the only approved drug commercially available for treatment of relapsed refractory CLL and Follicular Lymphoma (FL)[26]. In a phase 1 trial involving 54 heavily pretreated relapsed/refractory CLL patients, Idelalisib as a single agent had substantial activity with a 72% overall response rate (54% in patients with 17p deletion) with a median time to response of 1 month. As a continuous oral therapy, the drug was well tolerated and had a durable response with treatment extending > 3 years in some patients[27]. In a phase 2 open label trial of 125 patients with history of relapsed/refractory indolent BCL (Follicular, Marginal zone, lymphoplasmacytic and small lymphocytic

lymphoma) Idelalisib had similar activity as in CLL with ORR of 57% and an acceptable safety profile[28]. Based on these findings there are many clinical trials in various phases exploring the potential of PI3K inhibitors in the treatment of relapsed BCL (Table II).

Duvelisib (IPI- 145) is another novel PI3K inhibitor which inhibits both delta and gamma isoform of PI3K. In a phase 1 trial involving 32 patients with indolent BCL, duvelisib demonstrated an ORR of 65% (with 25% CR) and was well tolerated [29].

Spleen Tyrosine Kinase (SYK) Inhibitor

SYK is another proximal kinase critically involved in the BCR pathway promoting cell proliferation and survival. Inhibition of SYK has been shown to abrogate BCR signaling and induce apoptosis[30]. Overexpression of SYK demonstrated in various BCL's is believed to be one of the underlying aberrations responsible for malignant transformation of B lymphocytes and provided additional rationale for targeting SYK[31]. Fostamatinib competitively inhibits SYK in-vitro and down regulates BCR signaling. In a phase 1/2 clinical trial, oral Fostamatinib has shown both safety and efficacy in a heavily pretreated relapsed refractory BCL cohort of 68 patients. Overall rates of response seen in CLL/SLL were 55%, DLBCL 22%, FL 10% and MCL 11%. Toxicity was mild and included diarrhea, fatigue and cytopenia [32].

The Phenomenon of "Redistribution Lymphocytosis"

Patients treated with Ibrutinib (BTK inhibitor), PI3K and SYK inhibitors often show rapid reduction of lymphadenopathy that is accompanied by peripheral lymphocytosis. This phenomenon known as "redistribution lymphocytosis" represents a compartmental shift of malignant lymphocytes from the bone marrow and lymph nodes into the peripheral circulation[15, 33]. In most patients, initial brisk lymphocytosis is transient and is followed by a return to pre-treatment baseline values usually within few months (< 8 months).

Table II: PI3K Inhibitor studies

	Phase	N	Histology	ORR	Study
Idelalisib + rituximab	1 / 2	30	Indolent NHL (iNHL)	77%	Fowler et al.[44]
Idelalisib + rituximab	3	390	CLL	Ongoing	NCT011539512
Idelalisib + bendamustine +/- rituximab	1 / 2	46	iNHL	77%-85%	Fowler et al.[44]
Duvelisib	2	TBD	iNHL	Ongoing	NCT01882803

However in certain patients lymphocytosis may be persistent lasting > 12 months [34]. It is important to recognize this phenomenon and not interpret it as progressive disease or treatment failure. Interestingly extended follow-up studies have actually suggested a favorable prognosis in patients exhibiting persistent lymphocytosis[34].

Future trends

The elucidation of the components of the BCR signaling pathway has allowed the creation of small molecule inhibitors that target specific constituents of this critical pathway. Despite the demonstration of efficacy by multiple agents targeting the BCR pathway, susceptibility to these agents is not uniform across all BCL histological subtypes. As evidenced by the differential response to ibrutinib in DLBCL of ABC type versus the GBC type, there is much yet to learn about these signaling pathways. The molecular basis of this difference offers the possibility of identifying new targets for drug development. The long term toxicity profile, mechanisms of resistance and degree of immunosuppression are largely unknown. The potential mechanisms of resistance include the acquisition of mutations in CARD11, BTK binding residue mutations and activation of alternate pathways that might bypass BCR signaling[35]. Thus early identification by molecular testing of resistance to BCR inhibitors would avoid prescription of ineffective therapy. However, given the advantage of oral availability, mild toxicity profile and unique mechanism of action of these drugs, they are particularly attractive for combination with other anti-cancer agents. The ideal combinations would be synergistic leading to reduced toxicity and a higher complete response rate ultimately improving overall survival. There are preclinical data targeting multiple kinases within BCR pathway that suggests augmented antitumor activity [36]. Several studies are underway exploring these options[37, 38]. Several small studies involving the combination of Idelalisib and Ibrutinib plus bendamustine with or without rituximab have shown efficacy and safety in the treatment of relapsed/refractory indolent BCL[39].

In addition to drugs targeting BCR signaling there are other novel agents being developed or integrated into the treatment of BCL. Agents such as ABT-199 targeting BCL2, Nivolumab an anti-PD1 receptor antibody, and SGN CD19A an anti-CD19 antibody drug conjugate are actively being studied either as single agents or in combination with existing drugs for treatment of BCL[40, 41, 42]. These novel agents are particularly attractive to combine with BCR pathway inhibitors due to their different mechanisms of action and offer the possibility of eliminating traditional chemotherapy and its attendant toxicity from the treatment paradigm. With the rapid expansion of our therapeutic armamentarium an integrated approach to therapy based upon traditional clinical trial methodology might be outpaced by our understanding of molecular events upon which these diseases are dependent. Thus such platforms as next generation sequencing might allow a more definitive prediction of efficacy of not only single agents but also their rational combination. It behooves the informed practitioner to stay abreast of these advances as the design of treatment strategy becomes ever the more individualized.

References:

1. Wolf M, Mathews JP, Stone J, Cooper IA, Robertson TI, Fox RM. Long-term survival advantage of MACOP-B over CHOP in intermediate-grade non-Hodgkin's lymphoma: the Australian and New Zealand Lymphoma Group. Ann Oncol 1997; 8: 71-75.

2. Fisher RI, Gaynor ER, Dahlberg S, Oken MM, Grogan TM, Mize EM. et al. Comparison of a standard regimen (CHOP) with three intensive chemotherapy regimens for advanced non-Hodgkin's lymphoma. N Engl J Med 1993; 328:1002-1006; http://dx.doi.org/10.1056/NEJM199304083281404

3. Mclaughlin P, Grillo-Lopez AJ, Link BK, Levy R, Czuczman MS, Williams ME, J Lister et al. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four dose treatment program. JCO 1998 August Vol 16(8) 2825-33.

4. Shan D, Ledbetter JA, Press OW. Apoptosis of malignant human B cells by ligation of CD20 with monoclonal antibodies. Blood 1998; 91(5):1644–1652.

5. Feugier P, Van Hoof A, Sebban C, Solal-Celigny P, Bouabdallah R, Ferme C et al: Long-term results of the R-CHOP study in the treatment of elderly patients with diffuse large B-cell lymphoma: A study by the Groupe d'Etude des Lymphomes de l'Adulte. J Clin Oncol 2005; 23(18):4117-4126; http://dx.doi.org/10.1200/JCO.2005.09.131

6. Gisselbrecht C, Glass B, Mounier N, Gill DS, Linch DC, Trneny M et al. Salvage regimens with autologous transplantation for relapsed large B cell lymphoma in the rituximab era. JCO Sep 20, 2010 vol 28. No.27 4184-4190; http://dx.doi.org/10.1200/JCO.2010.28.1618

7. Zhang M, Srivatsava G, Lu L. The pre-B cell receptor and its function during B cell development. Cell Mol Immunology 2004 Apr; 1(2):89-94.

8. Dal Porto JM, Gauld SB, Merrell KT, Mills D, Pugh-Bernard AE, Cambier J. B cell antigen receptor signaling 101. Mol. Immunol 2004. 41(6-7), 599–613; http://dx.doi.org/10.1016/j.molimm.2004.04.008

9. Davis RE, Ngo VN, Lenz G, Tolar P, Young RM, Romesser PB et al. Chronic active B- cell receptor signaling in diffuse large B-cell lymphoma. Nature 2010 Jan 7; 463(7277):88-92; http://dx.doi.org/10.1038/nature08638

10. Neimann CU, Wiestner A. B-cell receptor signaling as a driver of lymhoma development and evolution. Seminal Cancer Biol 2013 Dec; 23(6):410-2.

11. Niiro H, Clark EA: Regulation of B-cell fate by antigen-receptor signals. Nat Rev Immunol 2002, 2: 945-956.

12. Qui Y, Kung H. Signaling network of the Btk family kinases. Oncogene. 2000(19); 5651-5661.

13. Honiberg LA, Smith AM, Sirsawada M, Verner E, Loury D, Chang B, et al. The Btk inhibitor PCI-3276 blocks B cell activation and is efficacious in models of autoimmune disease and B cell malignancy. Proc Natl Acad Sci USA 2010; 107:13075-13080; http://dx.doi.org/10.1073/pnas.1004594107

14. Wang ML, Rule S, Martin P, Goy A, Auer R, Kahl BS et al. Targeting BTK with Ibrutinib in Relapsed or Refractory Mantle-Cell Lymphoma. N Engl J Med 2013; 369:507-516; http://dx.doi.org/10.1056/NEJMoa1306220

15. Byrd JC, Furman RR, Coutre SE, Flinn IW, Burger JA, Blum KA et al. Targeting BTK with Ibrutinib in Relapsed Chronic Lymphocytic Leukemia N Engl J Med 2013; 369: 32-42; http://dx.doi.org/10.1056/NEJMoa1215637

16. Wilson WH, Gerecitano JF, Goy A, Sven de Vos, Kenkre PV, Barr PM et al. The Bruton's Tyrosine Kinase (BTK) Inhibitor, Ibrutinib (PCI-32765), Has Preferential Activity in the ABC Subtype of Relapsed/Refractory De Novo Diffuse Large B-Cell Lymphoma (DLBCL): Interim Results of a Multicenter, Open-Label, Phase 2 Study. Blood (ASH annual meeting abstracts) 2012;120(2):686.

17. Davis RE, Ngo VN, Lenz G, Tolar P, Young RM, Romesser PB, et al. Chronic active B-cell-receptor signalling in diffuse large B-cell lymphoma. Nature 2010 Jan 7; 463(7277):88-92; http://dx.doi.org/10.1038/nature08638
18 Treon SP, Xu L, Yang G, Zhou Y, Liu X, Cao Y et al. MYD88L265LP Somatic Mutation in Waldenstrom's Macroglobulinemia. N Engl J Med 2012; 367:826-833; http://dx.doi.org/10.1056/NEJMoa1200710
19. Treon SP, Tripsas CK, Yang G, Cao Y, Xu L, Hunter Z, et al: Ibrutinib in previously treated Waldenstrom's macroglobulinemia. N Engl J Med 2015; 372:1430-1440; http://dx.doi.org/10.1056/NEJMoa1501548
20. Brown JR, Sharman JP, Harb WA, Kelly KR, Schreeder MT, Sweetenham JW, et al. Phase Ib trial of AVL-292, a covalent inhibitor of Bruton's tyrosine kinase (Btk), in chronic lymphocytic leukemia (CLL) and B-non-Hodgkin lymphoma (b-NHL). ASCO Meeting Abstracts 2012; 30:8032.
21. Kozaki R, Yoshizawa T, Yasuhiro T, Mirjolet J, Birkett J, Narita M, et al. Development of a Bruton's tyrosine kinase (Btk) inhibitor–ONO-WG-307, a potential treatment for B-cell malignancies. Cancer Res 2012; 72(8 Suppl): Abstract nr 857; http://dx.doi.org/1538-7445.AM2012-857
22 Wymann MP, Zvelebil M, Laffargue M. Phosphoinositide 3-kinase signaling – which way to target? *Trends Pharmacol Sci*. 2003; 24(7):366-376; http://dx.doi.org/3748/wjg.v20.i41.15190
23. Engelman JA, Luo J, Cantley LC. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. Nat Rev Genet 2006(7): 606-619; http://dx.doi.org/10.1038/nrg1879
24 Schmid MC, Avraamides CJ, Dippold HC, Franco I, Foubert P, Ellies LG, et al. Receptor tyrosine kinases and TLR/IL1Rs unexpectedly activate myeloid cell PI3K gamma, a single convergent point promoting tumor inflammation and progression. Cancer Cell 2011;19(6):715-727; http://dx.doi.org/10.1016/j.ccr.2011.04.016
25. Hoellenriegel J, Meadows SA, Sivina M, Wierda WG, Kantarjian H, Keating MJ, et al. The phosphoinositide 3 -kinase delta inhibitor CAL-101, inhibitis B-cell receptor signaling and chemokine networks in chronic lymphocytic leukemia. Blood 2011;118(13):3603-3612; http://dx.doi.org/10.1182/blood-2011-05-352492
26. Flinn IW, Kahl BS, Leonard JP, Furman RR, Brown JR, Byrd JC. Idelalisib, a selective inhibior of phosphatidylinositol 3-kinase - δ, as therapy for previously treated indolent non-hodgkin lymhoma. Blood 2014; 123 (22) 3406-3413; http://dx.doi.org/10.1182/blood-2103-11-538546
27. Brown JR, Byrd JC, Coutre SE, Benson DM, Flinn IW, Wagner-Johnston ND, et al. Idelalisib, an inhibitor of phosphatidylinositol 3-Kinase p110δ, for relapsed /refractory chronic lymphocytic leukemia. Blood 2014 :123(22) 3390-3397; http://dx.doi.org/10.1182/blood-2013-11-535047
28. Gopal AK, Kahl BS, de Vos S, Wagner-Johnston ND, Schuster SJ, Jurczak WJ, et al. PI3Kδ inhibition by idelalisib in patients with relapsed indolent lymphoma. N Engl J Med 2014 Mar 13;370(11):1008-18; http://dx.doi.org/10.1056/NEJMoa1314583
29. Flinn I, Oki Y, Patel M, Horowitz SM, Foss FM, Sweeney J, Kerstin Allen MA, et al. A phase 1 evaluation of Duvelisib (IPI-145), a PI3K-δ,γ Inhibitor, in Patients with Relapsed/Refractory iNHL. ASH Annual Meeting (2014) Abstract - 802.
30. Mocsai A, Ruland J, Tybulewicz VL. The SYK tyrosine kinase: a crucial plyer in diverse biological functions. Nat Rev

Immunol. 2010;10(6):387-402; http://dx.doi.org/10.1038/nri2765
31. Hoellenreigel J, Coffey GP, Sinha U, Pandey A, Sivina M, Ferrajoli A, et al. Selective, novel spleen tyrosine kinase (syk) inhibitors suppress chronic lymphocytic leukemia B -cell activation and migration. Leukemia. 2012;26(7):1576-1583; http://dx.doi.org/10.1038/leu.2012.24
32. Friedberg JW, Sharman J, Sweetenham J, Johnston PB, Vose JM, LaCasce A, et al. Inhibition of Syk with fostamatinib disodium has significant clinical activity in non-Hodgkin lymphoma and chronic lymphocytic leukemia Jonathan. Blood 2010; 115(13): 2578-2585; http://dx.doi.org/10.1182/blood-2009-08-236471
33. Burger JA, Montserrat E. Coming full circle: 70 years of chronic lymphocytic leukemia cell redistribution, from glucocorticoids to inhibitors of B-cell receptor signaling. Blood 2013; 121(9): 1501-1509; http://dx.doi.org/10.1182/blood-2012-08-452607
34 Rossi D, Gaidano G. Lymphocytosis and inbrutinib treatment of CLL. Blood 2014; 123(12): 1772-1774; http://dx.doi.org/10-1182/blood-2014-01-549493
35. Fowler N, David C. Targeting B-cell receptor signaling: Changing the paradigm. ASH education book 2013; 2013(1): 553- 560.
36. O'Brien SM, Barrientos JC, Flinn IW, Barr PM, Burger JA, Navarro T, et al. Combination of the Bruton's tyrosine kinase (BTK) inhibitor PCI-32765 with bendamustine (B)/ rituximab (R) (BR) in patients with relapsed refractory chronic lymphocytic leukemia (CLL): Interim analysis of pahse 1b?II study. ASCO Meeting Abstracts 2012; 30:6515.
37. A phase 1/1b safety and eficacy study of the PI#K-delta inhibitor TGR-1202 and ibrutinib in patients with CLL or MCL. NCT02268851.
38. A phase 2 of GS-9973 (syk inhibitor) in combination with Idelalisib in subjects with relapsed or refractory hematologic malignancies. NCT01796470.
39. Blum KA, Christian B, Flyn JM, Jaglowski SM, Jones JA, Maddocks K, et al. A phase I trial of the Bruton's tyrosine kinase (BTK) inhibitor, ibrutinib (PCI-32765), in combination with rituximab (R) and benadmustine in patients with relapsed/refractory non-non-Hodgkin's lymhoma (NHL). Blood (ASH Annual meeting). Abstract 2012; 120(21):1643.
40. Souers AJ, Leverson JD, Boghaert ER, Ackler SL, Catron ND, Chen J et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. Nat Med 2013; 19(2): 202-208; http://dx.doi.org/10.1038/nm.3048
41. Lesokhin AM, Ansell SM, Armand P, Scott EC, Halwani A, Gutierrez M, Millenson MM, et al. 291 preliminary results of a phase I study of Nivolumab (BMS-936558) in patients with relapsed or refractory lymphoid malignancy. ASH Annual Meeting 2014. Abstract 624.
42. Forero- Torres A, Moskowitz C, Advani RH, Shah BD, Kostic A, Albertson TM, Sandalic L, et al. Interim analysis of a phase 1, open -label, dose-escalation study of SGN-CD19A in patients with relapsed or refractory B-lineage non-hodgkin lymphoma (NHL). J Clin Oncol 2014; 32(5s) (suppl: abstr 8505).
43. Younes A, Flinn I, Berdeja J, Friedberg J, Alberti S. Phase Ib study combining ibrutinib with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) in patients with CD-20 positive B-cell non-Hodgkin lymphoma (NHL). ASCO Meeting Abstracts 2012; 30:8502.
44. Fowler NH, deVos S, Schreeder MT, et al. Combination of phosphatidylinositol 3-kinase delta (PI3K delta) inhibitor, GS-1101 (CAL-101) with rituximab and/or bendamustine are tolerable and highly active in previously treated , indolent non-

Hodgkin lymphoma: results from a phase I study (Abstract). Blood (ASH annual Meeting Abstracts) 2012; 120(21): 3645.
45. Wang M, Hagemeister F, Westin JR, Fayad L, Samaneigo F, Turturro F et al. Ibrutinib and Rituximab are an efficacious and safe combination in relapsed Mantle cell lymphoma. Preliminary results from a Phase II clinical trial. ASH Abstracts 2014 ; 627.

Conflict of interests- Nil
Acknowledgements- Nil

Date of submission: 11-03-2015
Date of acceptance: 17-06-2015

Authors details:

1- **Corresponding author**: Clinical Assistant Professor, Division of Hematology and Cellular Therapy, Allegheny Health Network Cancer Institute, West Penn Hospital, 4800 Friendship Ave, Suite#2303, Pittsburgh , PA 15224. **E-mail :** ssadashi@wpahs.org

2- Chairman, Division of Hematology and Cellular Therapy, Allegheny Health Network Cancer Institute, West Penn Hospital, 4800 Friendship Ave, Suite#2303, Pittsburgh , PA 15224.