

# Bruton's Tyrosine Kinase (BTK)-Inhibitors in Cancer Therapeutics

Amarendranath Choudhury<sup>1,\*</sup> and Rudrarup Bhattacharjee<sup>2</sup>

<sup>1</sup>Department of Zoology, Patharkandi College, Karimganj-788724, Assam, India and <sup>2</sup>Adelaide Medical School, Faculty of Health and Medical Sciences, University of Adelaide, Adelaide, SA-5000, Australia

**Abstract:** While continuous global efforts are directed towards finding a conclusive medication for cancer treatment, any gold standard drug product or process is yet to be achieved. Although, many promising compounds were identified over the years to fight cancer progression, most of them still remain restricted within clinical trial phases. Among several identified pathways for modulating cancer progression, Bruton's tyrosine kinase (BTK) pathway inhibition has shown a greater potential. BTK regulates various aspects of B-cell lineages including proliferation, activation, differentiation, and survival. BTK is also responsible for multiple cellular signaling pathways, of which, FcR signaling cascade and B-cell receptor signaling are notable. Interestingly, BTK expression was reported to be excessively high in all the areas where B-cell mediated malignancies occur. Vivid involvement of BTK in several autoimmune diseases also rationally support the realization that BTK inhibition could be a conclusive therapeutic approach for cancer. In this short communication, we discuss the potential of BTK inhibitors in cancer therapeutics, considering the most recent literature.

**Keywords:** BTK, Inhibition, CLL, Ibrutinib, Cell.

## INTRODUCTION

Protein kinases refers to all the enzymes which are responsible for phosphorylating proteins using high-energy phosphate donors like Adenosine Triphosphate (ATP) and as such altering the structure and functionality of proteins [1]. Most of the kinase mediated covalent alterations are reversible, which has been evident from post-translational modification(s) of proteins and thereby effects protein functions [2]. Many studies have reported abnormal activation of protein kinases leading to malignancies. Such anomalies are also reported for alterations in cellular proliferation; metabolism; motility and survival along with angiogenesis and anti-tumor immune response evasion [3]. BTK mediated oncogenic signaling pathways also involve abnormal kinase activity, where BTK is essential for the survival of leukemia cells in B-cell mediated malignancies [4]. BTK is named after pediatrician, Ogdon Bruton who, in 1952, characterized the enzyme function in X-linked agammaglobulinemia (XLA). As per Bruton's description, the BTK is a non-receptor tyrosine kinase which is placed in the down-stream signal transduction pathway of B-cell receptor [5]. BTK's function has also been found to be essential for regulation of chemokine receptor, toll like receptor (TLR) and Fc- receptor signaling and it has been found abundantly expressing in myeloid lineage cells as well, as reviewed by Pal Singh et al. 2018 [6]. BTK is responsible for modulation of osteoclasts cells via receptor activator of nuclear factor-  $\kappa$ B. It also shows similar activity in CD32 signaling and NLRP3 inflammasome formation in blood cells [7,8,9]. Together, BTK plays a critical role in the regulation of tumor microenvironment, and hence, BTK inhibition could be a promising anticancer therapy for a broad-spectrum of oncogenic pathologies.

## BTK EXPRESSION AND ACTIVITY

In line with its key role in B-cell differentiation, survival, and proliferation, proper BTK activity is also

crucial for B cell homeostasis [10]. BTK recruitment to the cell membrane and subsequent downstream signaling is under tight control by various phosphate mediated mechanism. For example, a B-cell exclusive inhibitory receptor called Fc $\gamma$ RIIB contains immune tyrosine inhibitory motifs (ITIMs), which when phosphorylated, recruits SH-2 domain containing protein phosphatases (e.g., SHIP1) that dephosphorylates PIP3, which, in turn, inhibits the recruitment of BTK to the cell membrane, ultimately resulting in diminished BCR signalling [11, 12, 13]. Furthermore, several examples of negative regulation of BTK have been reported. For example, Protein Kinase C- $\beta$  or PKC $\beta$  phosphorylates S180 residue on BTK thereby altering its membrane localization [14]. Another protein called the iBTK directly inhibits BTK activity via binding to its PH domain [15]. Research also indicated that microRNA-185 downregulates BTK expression via reducing BTK mRNA levels. Similarly, other microRNAs, such as miR-210 and miR-425, also significantly reduce BTK expression [16]. The same study reported that these miRNAs show higher expression in primary Chronic Lymphocytic Leukemia (CLL) samples when treated with histone deacetylase (HDAC) inhibitors and consequently downregulates the BTK activity [16]. Apart from indirect regulatory pathways, BTK was found to show autoregulation via proteasome dependent positive feedback-loop whereby it stimulates self-transcription via NF- $\kappa$ B-dependent pathway resulting in increased BTK activity [17].

## BTK-INHIBITORS AND THEIR EFFICACY

### Ibrutinib

Ibrutinib (PCI-32765) is an oral BTK inhibitor and imparts its action via binding to the cysteine-481 (C481) residue at BTK kinase domain thereby causing irreversible kinase activity inhibition [18]. Ibrutinib was first approved in 2013 by FDA as a BTK inhibitor to be used in cancers and its in vivo effect was first

\*Address corresponding to this author at the Department of Zoology, Patharkandi College, Karimganj-788724, Assam, India; Email: amarendra.choudhury@gmail.com; Tel: +91 7003017920

demonstrated using an autoimmune disease mouse model and in dogs with spontaneous B-cell non-Hodgkin lymphoma, where it induced objective clinical responses [19, 20]. Ibrutinib's efficacy was first reported in a clinical study in patients with various relapsed/refractory B-cell malignancies, demonstrating clinical safety and promising long-term response, particularly in CLL and Mantle Cell Lymphoma (MCL). Patients responding to the therapy experienced a sustained reduction in lymphadenopathy, along with a transient increase in absolute lymphocyte count (i.e., lymphocytosis) [21]. The follow-up multicenter phase Ib/II trial, on relapsed/refractory CLL patients, consisting of a continuous ibrutinib regimen did show lymphocytosis in the initial weeks of treatment, however, after extended treatment, the lymphocyte counts were found to be normalized or even below baseline levels. However, treatment response rate, irrespective of clinical or genomic hazard factor, was about 71% [22]. In a Phase II clinical trial, ibrutinib was shown to be 68% effective in patients with refractory/relapsed MCL [23]. Ibrutinib has also been found to be effective in 38% of patients with recurrent/refractory Follicular Lymphoma (FL) [21] and its efficacy has been also demonstrated in Primary Central Nervous System Lymphoma (PCNSL), where 77% of patients showed response to the therapy [24]. Recent follow-up results from Phase III RESONATE-2 trial showed efficiency of ibrutinib in significantly increasing progressive free survival (PFS) rate of CLL patients [25]. With all the positive data at hand, it has been of interest to explain ibrutinib's method of therapeutic action. Ibrutinib was found to reduce survival of CLL cells (having CD40 or BCR) through repealing the ERK, PI3K and NF- $\kappa$ B signaling network [26]. Chemokine mediated CLL cell migration was also found to be impeded by ibrutinib, a mechanism advocating prevention of homing and retention of tumors in their niche of survival [27]. Ibrutinib shows its therapeutic effect also by modulating cell adhesion and consequent tumor microenvironment destabilization, for which one example being the ibrutinib induced impairment of CLL-cell attachment, to both fibronectin and VCAM1, mediated via integrin  $\alpha$ 4 $\beta$ 1 [28]. The examples cited here, put forward a dual action mechanism of ibrutinib, where on one hand, it disrupts B cell signaling pathways to jeopardize cancer cell proliferation and survival, and on the other hand, it interrupts the tumor cell stabilization via impairing their ability to interact with tumor microenvironment. Interestingly, all these positive effects of ibrutinib are available as a chemotherapy-free treatment regimen where the level of side-effects are also much more manageable, reviewed in Wen et al. 2021 [29].

Due to its chemotherapy-free nature, ibrutinib has not been associated with tumor lysis syndrome, a common complication in cancer due to cytotoxic chemotherapy resulting in metabolic irregularities due to rapidly dying tumor cells. In this context, ibrutinib's hinderance of B-cells from nourishing affected tissue niches, via inhibition of integrin-mediated leukemia cell retention, comes out as a more plausible anti-tumor mechanism rather than active hindrance towards

survival of only malignant B-cells [30]. Ibrutinib was recently shown to be effective in improving the functionality as well as yield of Chimeric Antigen receptor (CAR) T cells from CLL patients [31].

While ibrutinib does enjoy a massive clinical success as a BTK inhibitor, its curative potential in B-cell malignancies is still to be demonstrated, majorly owing to the fact that ibrutinib mostly gets prescribed as a lifelong therapy. This situation has given rise to selection and growth of therapy resistant cancer cell clones and so far, few mechanisms of resistance have already been described, as reviewed in Pal Singh et al. 2018 [6]. Thus, the promising mode of action of ibrutinib needs to be utilized to its full potential via utilizing this with other combinatorial treatment regimens, solely to minimize the prolonged treatment associated cancer cell resistance.

## Acalabrutinib

Another second-generation selective BTK inhibitor (also called ACP-196) with irreversible targeting and much lower off-target kinase activity, as shown in two mouse models of CLL [32]. It imparts this action via irreversible binding to C481 residue of BTK kinase domain but, avoids similar targeting in case of other kinases like EGFR, ITK, TXK, SRC family kinases, and JAK3. A canine model of Non-Hodgkin B-cell lymphoma was used for the first preclinical trial which demonstrated that acalabrutinib is more potent than ibrutinib in-vivo [20]. With a median follow-up of 14 months, the overall response rate in a phase I/II clinical trial in patients with relapsed/refractory CLL was found to be approximately 95% [33]. Notably, A phase III clinical trial (NCT02477696) with relapsed/refractory CLL patient participants is still underway (estimated completion June 2022) which aims to directly compare the PFS for acalabrutinib and ibrutinib. Talking about MCL patients, acalabrutinib was found to show about 81% response rate (with around 40% showing complete response) and hence got a fat-track approval by FDA for use in MCL patients [34, 35].

## BGB-3111

The BTK inhibitor BGB-311 has been shown to inhibit the proliferation of several MCL and Diffuse Large B-cell Lymphoma (DLBCL) cell lines, while having better selectivity against target and also better oral bioavailability compared to ibrutinib. A phase I/II clinical trial treated 45 CLL patients with BGB-3111 and reported it to be well tolerated by patients. Moreover, a follow-up after 7.5 months showed that 90% patients showed a response rate while there was no case of Richter Transformation as well [36].

## Ono/GS-4059

The compound was first used in a xenograft model of activated-B-cell-like (ABC)-DLBCL where the in-vivo efficacy was demonstrated. The anti-proliferative properties of this compound were demonstrated in-vitro using cell lines from DLBCL, FL, MCL and CLL [37]. In

patients with diverse B cell malignancies, early-phase clinical trial data shows varied response in relation to patient genetics, which has been reviewed in detail by Pal Singh et al., 2018 [6].

### BTK inhibition and Future Therapeutics

BTK mediated oncogenesis in solid tumors like ovarian, colorectal, prostate and brain has gained much pre-clinical evidence, and as such many phases I/II clinical trials have since been started for BTK inhibition in Solid Tumors [38, 39, 40]. The role of BTK inhibitor in modulating tumor microenvironment has made it promising even in BTK-negative solid tumors. While monotherapy with BTK-inhibitors shows only limited survival improvement in breast and pancreatic cancer, its combination with chemotherapy and immunotherapy showed significant increase in survival rate [41, 42]. As ibrutinib exhibited off-target inhibitory effect on JAK3, ITK and EGFR [19, 32], it may be employed as a supplement with immunotherapy in T-cell modulatory tumors without BTK as target [43]. The approach showed that combining ibrutinib in such cases of CLL or MCL enhances persistence of cells while decreasing co-inhibitors in the surface of CART Cells [31, 43, 44]. While ITK inhibition in T-cells, somewhat contradictorily, may benefit certain cancer therapeutic outcome, via enhanced immune memory formation through Th1 mediated CD4+ T cells, ultimately enhancing the CD8+ T cell mediated immunity against tumor [45]. While this remains a paradoxical good side of the non-specific targeting by BTK inhibitor, new approaches aimed towards more specific BTK targeting may, unfortunately forfeit these secondary good effects of off-targeting. However, as the knowledgebase evolves, a balance

needs to be stricken towards specific BTK inhibition and other forms of combination with them to eliminate the risk of tumor survival based on non-BTK mediated kinase activities.

### CONCLUSION

BTK targeting has shown to be an impressive therapeutic area in clinical trials for various B cell malignancies, particularly owing to its central role in several B-cell mediated signaling pathways, especially the BCR. In recent years, many advances have been made towards defining the complex BTK inhibition mechanisms. These include intrinsic signalosome pathways which being disrupted interferes with tumor cell survival, proliferation as well as migration [46]. BTK inhibition also interferes with tumor microenvironment retention via modulating crucial immune cells in that microenvironment. While Ibrutinib has been an excellent candidate as BTK inhibitor to treat malignancies, poor availability of the inhibitor treated myeloid cells makes it difficult to ascertain that such anti-cancer effects are specifically due to BTK inhibition or certain off-target effects of the compound on CD4+/CD8+ cells. It has been found that Ibrutinib represses the immunosuppressive properties of malignant cells in CLL via both BTK-dependent and independent pathways (e.g., via T-cell modulation) [47] and thus it becomes more crucial to identify that whether similar levels of anti-tumor efficacy can be seen with only BTK inhibition alone (without any off-target effects). However, it will be very interesting to see the use of BTK-inhibitors having additional specificity for associated kinases against certain malignancies. While BTK inhibition remains a very effective single agent therapy, there has been

**Table 1:** Important BTK Inhibitors against different cancer types with Patient Survival Rate.

BTK Inhibitor	Disease used for	Survival	References
Ibrutinib	Relapsed/Refractory CLL	At 12 months: 90% At 30 months: 87%	[48] [49]
	Treatment naïve CLL	At 24 months: 98% At 30 months: 96%	[49] [50]
	Relapsed/Refractory MCL	At 18 Months: 58%	[23]
Acalabrutinib (ACP-196)	Relapsed/Refractory CLL	At 16 Months: ~90%	[33]
	Relapsed/Refractory MCL	At 12 Months: 87%	[34]
Rituximab and Ibrutinib Combination	Waldenström macroglobulinemia (WM)	At 30 months: 94%	[51]
Venetoclax and Ibrutinib Combination	Treatment naïve CLL	Still not reported	[52]
Zanubrutinib (BGB-3111)	Relapsed/refractory B-cell malignancies	At 12 Months: 100% (PFS)	[53]
GS-4059 (ONO-4059)	Mature B cell malignancies	Only PFS: CLL: 874 days, MCL: 341 days, DLBCL: 54 days	[37]

evidences of development of resistance against it. Thus, a range of research studies have now been conducted to develop effective combination therapies to improve the clinical response in BTK-inhibitor resistant malignancy. There is still a gap of direct comparison between the efficacy and toxicity of available BTK inhibitors. Taking this situation into account, the therapeutic strategies using BTK inhibitors, at this point in time, needs to be designed based on the particular malignancy and patient subgroups, by using extensive analysis of clinical responses, development of resistance, their toxicity and of course, the quality of life for the patient being treated.

## Conflict of Interest

None

## Acknowledgement

My sincere thanks are due to Patharkandi College authority for providing sufficient support for this manuscript.

## REFERENCES

- [1] Manning, G., Whyte, D. B., Martinez, R., Hunter, T., & Sudarsanam, S. (2002). The protein kinase complement of the human genome. *Science (New York, N.Y.)*, 298(5600), 1912–1934. <https://doi.org/10.1126/science.1075762>
- [2] Berg JM, Tymoczko JL, Stryer L. Biochemistry. 5th edition. New York: W H Freeman; 2002. Section 10.4, Covalent Modification Is a Means of Regulating Enzyme Activity. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK22399/>
- [3] Gross, S., Rahal, R., Stransky, N., Lengauer, C., & Hoeflich, K. P. (2015). Targeting cancer with kinase inhibitors. *The Journal of clinical investigation*, 125(5), 1780–1789. <https://doi.org/10.1172/JCI176094>
- [4] Buggy, J. J., & Elias, L. (2012). Bruton tyrosine kinase (BTK) and its role in B-cell malignancy. *International reviews of immunology*, 31(2), 119–132. <https://doi.org/10.3109/08830185.2012.664797>
- [5] Hendriks, R. W., Yuvaraj, S., & Kil, L. P. (2014). Targeting Bruton's tyrosine kinase in B cell malignancies. *Nature reviews. Cancer*, 14(4), 219–232. <https://doi.org/10.1038/nrc3702>
- [6] Pal Singh, S., Dammeijer, F., & Hendriks, R. W. (2018). Role of Bruton's tyrosine kinase in B cells and malignancies. *Molecular cancer*, 17(1), 57. <https://doi.org/10.1186/s12943-018-0779-z>
- [7] Shinohara, M., Koga, T., Okamoto, K., Sakaguchi, S., Arai, K., Yasuda, H., Takai, T., Kodama, T., Morio, T., Geha, R. S., Kitamura, D., Kurosaki, T., Ellmeier, W., & Takayanagi, H. (2008). Tyrosine kinases Btk and Tec regulate osteoclast differentiation by linking RANK and ITAM signals. *Cell*, 132(5), 794–806. <https://doi.org/10.1016/j.cell.2007.12.037>
- [8] Oda, A., Ikeda, Y., Ochs, H. D., Druker, B. J., Ozaki, K., Handa, M., Ariga, T., Sakiyama, Y., Witte, O. N., & Wahl, M. I. (2000). Rapid tyrosine phosphorylation and activation of Bruton's tyrosine/Tec kinases in platelets induced by collagen binding or CD32 cross-linking. *Blood*, 95(5), 1663–1670
- [9] Ito, M., Shichita, T., Okada, M., Komine, R., Noguchi, Y., Yoshimura, A., & Morita, R. (2015). Bruton's tyrosine kinase is essential for NLRP3 inflammasome activation and contributes to ischaemic brain injury. *Nature communications*, 6, 7360. <https://doi.org/10.1038/ncomms8360>
- [10] Geier, C. B., Sauerwein, K., Leiss-Piller, A., Zmek, I., Fischer, M. B., Eibl, M. M., & Wolf, H. M. (2018). Hypomorphic Mutations in the BCR Signalosome Lead to Selective Immunoglobulin M Deficiency and Impaired B-cell Homeostasis. *Frontiers in immunology*, 9, 2984. <https://doi.org/10.3389/fimmu.2018.02984>
- [11] Muta, T., Kurosaki, T., Misulovin, Z., Sanchez, M., Nussenzweig, M. C., & Ravetch, J. V. (1994). A 13-amino-acid motif in the cytoplasmic domain of Fc gamma RIIB modulates B-cell receptor signalling. *Nature*, 368(6466), 70–73. <https://doi.org/10.1038/368070a0>
- [12] Bolland, S., & Ravetch, J. V. (1999). Inhibitory pathways triggered by ITIM-containing receptors. *Advances in immunology*, 72, 149–177. [https://doi.org/10.1016/s0065-2776\(08\)60019-x](https://doi.org/10.1016/s0065-2776(08)60019-x)
- [13] Ono, M., Bolland, S., Tempst, P., & Ravetch, J. V. (1996). Role of the inositol phosphatase SHIP in negative regulation of the immune system by the receptor Fc(gamma)RIIB. *Nature*, 383(6597), 263–266. <https://doi.org/10.1038/383263a0>
- [14] Kang, S. W., Wahl, M. I., Chu, J., Kitaura, J., Kawakami, Y., Kato, R. M., Tabuchi, R., Tarakhovskiy, A., Kawakami, T., Turck, C. W., Witte, O. N., & Rawlings, D. J. (2001). PKCbeta modulates antigen receptor signaling via regulation of Btk membrane localization. *The EMBO journal*, 20(20), 5692–5702. <https://doi.org/10.1093/emboj/20.20.5692>

- [15] Liu, W., Quinto, I., Chen, X., Palmieri, C., Rabin, R. L., Schwartz, O. M., Nelson, D. L., & Scala, G. (2001). Direct inhibition of Bruton's tyrosine kinase by IBtk, a Btk-binding protein. *Nature immunology*, 2(10), 939–946. <https://doi.org/10.1038/ni1001-939>
- [16] Bottoni, A., Rizzotto, L., Lai, T. H., Liu, C., Smith, L. L., Mantel, R., Reiff, S., El-Gamal, D., Larkin, K., Johnson, A. J., Lapalombella, R., Lehman, A., Plunkett, W., Byrd, J. C., Blachly, J. S., Woyach, J. A., & Sampath, D. (2016). Targeting BTK through microRNA in chronic lymphocytic leukemia. *Blood*, 128(26), 3101–3112. <https://doi.org/10.1182/blood-2016-07-727750>
- [17] Yu, L., Mohamed, A. J., Simonson, O. E., Vargas, L., Blomberg, K. E., Björkstrand, B., Arteaga, H. J., Nore, B. F., & Smith, C. I. (2008). Proteasome-dependent autoregulation of Bruton tyrosine kinase (Btk) promoter via NF-kappaB. *Blood*, 111(9), 4617–4626. <https://doi.org/10.1182/blood-2007-10-121137>
- [18] Pan, Z., Scheerens, H., Li, S. J., Schultz, B. E., Sprengeler, P. A., Burrill, L. C., Mendonca, R. V., Sweeney, M. D., Scott, K. C., Grothaus, P. G., Jeffery, D. A., Spoerke, J. M., Honigberg, L. A., Young, P. R., Dalrymple, S. A., & Palmer, J. T. (2007). Discovery of selective irreversible inhibitors for Bruton's tyrosine kinase. *ChemMedChem*, 2(1), 58–61. <https://doi.org/10.1002/cmdc.200600221>
- [19] Honigberg, L. A., Smith, A. M., Sirisawad, M., Verner, E., Loury, D., Chang, B., Li, S., Pan, Z., Thamm, D. H., Miller, R. A., & Buggy, J. J. (2010). The Bruton tyrosine kinase inhibitor PCI-32765 blocks B-cell activation and is efficacious in models of autoimmune disease and B-cell malignancy. *Proceedings of the National Academy of Sciences of the United States of America*, 107(29), 13075–13080. <https://doi.org/10.1073/pnas.1004594107>
- [20] Harrington, B. K., Gardner, H. L., Izumi, R., Hamdy, A., Rothbaum, W., Coombes, K. R., Covey, T., Kaptein, A., Gulrajani, M., Van Lith, B., Krejsa, C., Coss, C. C., Russell, D. S., Zhang, X., Urie, B. K., London, C. A., Byrd, J. C., Johnson, A. J., & Kisseberth, W. C. (2016). Preclinical Evaluation of the Novel BTK Inhibitor Acalabrutinib in Canine Models of B-Cell Non-Hodgkin Lymphoma. *PloS one*, 11(7), e0159607. <https://doi.org/10.1371/journal.pone.0159607>
- [21] Advani, R. H., Buggy, J. J., Sharman, J. P., Smith, S. M., Boyd, T. E., Grant, B., Kolibaba, K. S., Furman, R. R., Rodriguez, S., Chang, B. Y., Sukbuntherng, J., Izumi, R., Hamdy, A., Hedrick, E., & Fowler, N. H. (2013). Bruton tyrosine kinase inhibitor ibrutinib (PCI-32765) has significant activity in patients with relapsed/refractory B-cell malignancies. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*, 31(1), 88–94. <https://doi.org/10.1200/JCO.2012.42.7906>
- [22] Byrd, J. C., Furman, R. R., Coutre, S. E., Flinn, I. W., Burger, J. A., Blum, K. A., Grant, B., Sharman, J. P., Coleman, M., Wierda, W. G., Jones, J. A., Zhao, W., Heerema, N. A., Johnson, A. J., Sukbuntherng, J., Chang, B. Y., Clow, F., Hedrick, E., Buggy, J. J., James, D. F., ... O'Brien, S. (2013). Targeting BTK with ibrutinib in relapsed chronic lymphocytic leukemia. *The New England journal of medicine*, 369(1), 32–42. <https://doi.org/10.1056/NEJMoa1215637>
- [23] Wang, M. L., Rule, S., Martin, P., Goy, A., Auer, R., Kahl, B. S., Jurczak, W., Advani, R. H., Romaguera, J. E., Williams, M. E., Barrientos, J. C., Chmielowska, E., Radford, J., Stilgenbauer, S., Dreyling, M., Jdrzejczak, W. W., Johnson, P., Spurgeon, S. E., Li, L., Zhang, L., ... Blum, K. A. (2013). Targeting BTK with ibrutinib in relapsed or refractory mantle-cell lymphoma. *The New England journal of medicine*, 369(6), 507–516. <https://doi.org/10.1056/NEJMoa1306220>
- [24] Grommes, C., Pastore, A., Palaskas, N., Tang, S. S., Campos, C., Schartz, D., Codega, P., Nichol, D., Clark, O., Hsieh, W. Y., Rohle, D., Rosenblum, M., Viale, A., Tabar, V. S., Brennan, C. W., Gavrilovic, I. T., Kaley, T. J., Nolan, C. P., Omuro, A., Pentsova, E., ... Mellinghoff, I. K. (2017). Ibrutinib Unmasks Critical Role of Bruton Tyrosine Kinase in Primary CNS Lymphoma. *Cancer discovery*, 7(9), 1018–1029. <https://doi.org/10.1158/2159-8290.CD-17-0613>
- [25] Burger, J. A., Barr, P. M., Robak, T., Owen, C., Ghia, P., Tedeschi, A., Bairey, O., Hillmen, P., Coutre, S. E., Devereux, S., Grosicki, S., McCarthy, H., Simpson, D., Offner, F., Moreno, C., Dai, S., Lal, I., Dean, J. P., & Kipps, T. J. (2020). Long-term efficacy and safety of first-line ibrutinib treatment for patients with CLL/SLL: 5 years of follow-up from the phase 3 RESONATE-2 study. *Leukemia*, 34(3), 787–798. <https://doi.org/10.1038/s41375-019-0602-x>
- [26] Herman, S. E., Gordon, A. L., Hertlein, E., Ramanunni, A., Zhang, X., Jaglowski, S., Flynn, J., Jones, J., Blum, K. A., Buggy, J. J., Hamdy, A., Johnson, A. J., & Byrd, J. C. (2011). Bruton tyrosine kinase represents a promising therapeutic target for treatment of chronic lymphocytic leukemia and is effectively targeted by PCI-32765. *Blood*, 117(23), 6287–6296. <https://doi.org/10.1182/blood-2011-01-328484>

- [27] de Gorter, D. J., Beuling, E. A., Kersseboom, R., Middendorp, S., van Gils, J. M., Hendriks, R. W., Pals, S. T., & Spaargaren, M. (2007). Bruton's tyrosine kinase and phospholipase Cgamma2 mediate chemokine-controlled B cell migration and homing. *Immunity*, 26(1), 93–104. <https://doi.org/10.1016/j.immuni.2006.11.012>
- [28] de Rooij, M. F., Kuil, A., Geest, C. R., Eldering, E., Chang, B. Y., Buggy, J. J., Pals, S. T., & Spaargaren, M. (2012). The clinically active BTK inhibitor PCI-32765 targets B-cell receptor- and chemokine-controlled adhesion and migration in chronic lymphocytic leukemia. *Blood*, 119(11), 2590–2594. <https://doi.org/10.1182/blood-2011-11-390989>
- [29] Wen, T., Wang, J., Shi, Y., Qian, H., & Liu, P. (2021). Inhibitors targeting Bruton's tyrosine kinase in cancers: drug development advances. *Leukemia*, 35(2), 312–332. <https://doi.org/10.1038/s41375-020-01072-6>
- [30] Burger, J. A., & Wiestner, A. (2018). Targeting B cell receptor signalling in cancer: preclinical and clinical advances. *Nature reviews. Cancer*, 18(3), 148–167. <https://doi.org/10.1038/nrc.2017.121>
- [31] Fan, F., Yoo, H. J., Stock, S., Wang, L., Liu, Y., Schubert, M. L., Wang, S., Neuber, B., Hückelhoven-Krauss, A., Gern, U., Schmitt, A., Müller-Tidow, C., Dreger, P., Schmitt, M., & Sellner, L. (2021). Ibrutinib for improved chimeric antigen receptor T-cell production for chronic lymphocytic leukemia patients. *International journal of cancer*, 148(2), 419–428. <https://doi.org/10.1002/ijc.33212>
- [32] Herman, S., Montraveta, A., Niemann, C. U., Mora-Jensen, H., Gulrajani, M., Krantz, F., Mantel, R., Smith, L. L., McClanahan, F., Harrington, B. K., Colomer, D., Covey, T., Byrd, J. C., Izumi, R., Kaptein, A., Ulrich, R., Johnson, A. J., Lannutti, B. J., Wiestner, A., & Woyach, J. A. (2017). The Bruton Tyrosine Kinase (BTK) Inhibitor Acalabrutinib Demonstrates Potent On-Target Effects and Efficacy in Two Mouse Models of Chronic Lymphocytic Leukemia. *Clinical cancer research : an official journal of the American Association for Cancer Research*, 23(11), 2831–2841. <https://doi.org/10.1158/1078-0432.CCR-16-0463>
- [33] Byrd, J. C., Harrington, B., O'Brien, S., Jones, J. A., Schuh, A., Devereux, S., Chaves, J., Wierda, W. G., Awan, F. T., Brown, J. R., Hillmen, P., Stephens, D. M., Ghia, P., Barrientos, J. C., Pagel, J. M., Woyach, J., Johnson, D., Huang, J., Wang, X., Kaptein, A., ... Furman, R. R. (2016). Acabrutinib (ACP-196) in Relapsed Chronic Lymphocytic Leukemia. *The New England journal of medicine*, 374(4), 323–332. <https://doi.org/10.1056/NEJMoa1509981>
- [34] Wang, M., Rule, S., Zinzani, P. L., Goy, A., Casasnovas, O., Smith, S. D., Damaj, G., Doorduijn, J., Lamy, T., Morschhauser, F., Panizo, C., Shah, B., Davies, A., Eek, R., Dupuis, J., Jacobsen, E., Kater, A. P., Le Gouill, S., Oberic, L., Robak, T., ... Jurczak, W. (2018). Acabrutinib in relapsed or refractory mantle cell lymphoma (ACE-LY-004): a single-arm, multicentre, phase 2 trial. *Lancet (London, England)*, 391(10121), 659–667. [https://doi.org/10.1016/S0140-6736\(17\)33108-2](https://doi.org/10.1016/S0140-6736(17)33108-2)
- [35] Acabrutinib Approved for MCL. (2018). *Cancer discovery*, 8(1), OF6. <https://doi.org/10.1158/2159-8290.CD-NB2017-158>
- [36] Thompson, P. A., & Burger, J. A. (2018). Bruton's tyrosine kinase inhibitors: first and second generation agents for patients with Chronic Lymphocytic Leukemia (CLL). *Expert opinion on investigational drugs*, 27(1), 31–42. <https://doi.org/10.1080/13543784.2018.1404027>
- [37] Walter, H. S., Rule, S. A., Dyer, M. J., Karlin, L., Jones, C., Cazin, B., Quittet, P., Shah, N., Hutchinson, C. V., Honda, H., Duffy, K., Birkett, J., Jamieson, V., Courtenay-Luck, N., Yoshizawa, T., Sharpe, J., Ohno, T., Abe, S., Nishimura, A., Cartron, G., ... Salles, G. (2016). A phase 1 clinical trial of the selective BTK inhibitor ONO/GS-4059 in relapsed and refractory mature B-cell malignancies. *Blood*, 127(4), 411–419. <https://doi.org/10.1182/blood-2015-08-664086>
- [38] Kokabee, L., Wang, X., Sevinsky, C. J., Wang, W. L., Cheu, L., Chittur, S. V., Karimipoor, M., Tenniswood, M., & Conklin, D. S. (2015). Bruton's tyrosine kinase is a potential therapeutic target in prostate cancer. *Cancer biology & therapy*, 16(11), 1604–1615. <https://doi.org/10.1080/15384047.2015.1078023>
- [39] Grassilli, E., Pisano, F., Cialdella, A., Bonomo, S., Missaglia, C., Cerrito, M. G., Masiero, L., Ianzano, L., Giordano, F., Cicirelli, V., Narloch, R., D'Amato, F., Noli, B., Ferri, G. L., Leone, B. E., Stanta, G., Bonin, S., Helin, K., Giovannoni, R., & Lavitrano, M. (2016). A novel oncogenic BTK isoform is overexpressed in colon cancers and required for RAS-mediated transformation. *Oncogene*, 35(33), 4368–4378. <https://doi.org/10.1038/onc.2015.504>

- [40] Zucha, M. A., Wu, A. T., Lee, W. H., Wang, L. S., Lin, W. W., Yuan, C. C., & Yeh, C. T. (2015). Bruton's tyrosine kinase (Btk) inhibitor ibrutinib suppresses stem-like traits in ovarian cancer. *Oncotarget*, 6(15), 13255–13268. <https://doi.org/10.18632/oncotarget.3658>
- [41] Stiff, A., Trikha, P., Wesolowski, R., Kendra, K., Hsu, V., Uppati, S., McMichael, E., Duggan, M., Campbell, A., Keller, K., Landi, I., Zhong, Y., Dubovsky, J., Howard, J. H., Yu, L., Harrington, B., Old, M., Reiff, S., Mace, T., Tridandapani, S., ... Carson, W. E., 3rd (2016). Myeloid-Derived Suppressor Cells Express Bruton's Tyrosine Kinase and Can Be Depleted in Tumor-Bearing Hosts by Ibrutinib Treatment. *Cancer research*, 76(8), 2125–2136. <https://doi.org/10.1158/0008-5472.CAN-15-1490>
- [42] Gunderson, A. J., Kaneda, M. M., Tsujikawa, T., Nguyen, A. V., Affara, N. I., Ruffell, B., Gorjestani, S., Liudahl, S. M., Truitt, M., Olson, P., Kim, G., Hanahan, D., Tempero, M. A., Sheppard, B., Irving, B., Chang, B. Y., Varner, J. A., & Coussens, L. M. (2016). Bruton Tyrosine Kinase-Dependent Immune Cell Cross-talk Drives Pancreas Cancer. *Cancer discovery*, 6(3), 270–285. <https://doi.org/10.1158/2159-8290.CD-15-0827>
- [43] Fraietta, J. A., Beckwith, K. A., Patel, P. R., Ruella, M., Zheng, Z., Barrett, D. M., Lacey, S. F., Melenhorst, J. J., McGettigan, S. E., Cook, D. R., Zhang, C., Xu, J., Do, P., Hulitt, J., Kudchodkar, S. B., Cogdill, A. P., Gill, S., Porter, D. L., Woyach, J. A., Long, M., ... Maus, M. V. (2016). Ibrutinib enhances chimeric antigen receptor T-cell engraftment and efficacy in leukemia. *Blood*, 127(9), 1117–1127. <https://doi.org/10.1182/blood-2015-11-679134>
- [44] Ruella, M., Kenderian, S. S., Shestova, O., Fraietta, J. A., Qayyum, S., Zhang, Q., Maus, M. V., Liu, X., Nunez-Cruz, S., Klichinsky, M., Kawalekar, O. U., Milone, M., Lacey, S. F., Mato, A., Schuster, S. J., Kalos, M., June, C. H., Gill, S., & Wasik, M. A. (2016). The Addition of the BTK Inhibitor Ibrutinib to Anti-CD19 Chimeric Antigen Receptor T Cells (CART19) Improves Responses against Mantle Cell Lymphoma. *Clinical cancer research : an official journal of the American Association for Cancer Research*, 22(11), 2684–2696. <https://doi.org/10.1158/1078-0432.CCR-15-1527>
- [45] Dubovsky, J. A., Beckwith, K. A., Natarajan, G., Woyach, J. A., Jaglowski, S., Zhong, Y., Hessler, J. D., Liu, T. M., Chang, B. Y., Larkin, K. M., Stefanovski, M. R., Chappell, D. L., Frizzera, F. W., Smith, L. L., Smucker, K. A., Flynn, J. M., Jones, J. A., Andritsos, L. A., Maddocks, K., Lehman, A. M., ... Byrd, J. C. (2013). Ibrutinib is an irreversible molecular inhibitor of ITK driving a Th1-selective pressure in T lymphocytes. *Blood*, 122(15), 2539–2549. <https://doi.org/10.1182/blood-2013-06-507947>
- [46] Aalipour, A., & Advani, R. H. (2014). Bruton's tyrosine kinase inhibitors and their clinical potential in the treatment of B-cell malignancies: focus on ibrutinib. *Therapeutic advances in hematology*, 5(4), 121–133. <https://doi.org/10.1177/2040620714539906>
- [47] Long, M., Beckwith, K., Do, P., Mundy, B. L., Gordon, A., Lehman, A. M., Maddocks, K. J., Cheney, C., Jones, J. A., Flynn, J. M., Andritsos, L. A., Awan, F., Fraietta, J. A., June, C. H., Maus, M. V., Woyach, J. A., Caligiuri, M. A., Johnson, A. J., Muthusamy, N., & Byrd, J. C. (2017). Ibrutinib treatment improves T cell number and function in CLL patients. *The Journal of clinical investigation*, 127(8), 3052–3064. <https://doi.org/10.1172/JCI89756>
- [48] Byrd, J. C., Brown, J. R., O'Brien, S., Barrientos, J. C., Kay, N. E., Reddy, N. M., Coutre, S., Tam, C. S., Mulligan, S. P., Jaeger, U., Devereux, S., Barr, P. M., Furman, R. R., Kipps, T. J., Cymbalista, F., Pocock, C., Thornton, P., Caligaris-Cappio, F., Robak, T., Delgado, J., ... RESONATE Investigators (2014). Ibrutinib versus ofatumumab in previously treated chronic lymphoid leukemia. *The New England journal of medicine*, 371(3), 213–223. <https://doi.org/10.1056/NEJMoa1400376>
- [49] Coutre, S. E., Furman, R. R., Flinn, I. W., Burger, J. A., Blum, K., Sharman, J., Jones, J., Wierda, W., Zhao, W., Heerema, N. A., Johnson, A. J., Tran, A., Zhou, C., Bilotti, E., James, D. F., Byrd, J. C., & O'Brien, S. (2017). Extended Treatment with Single-Agent Ibrutinib at the 420 mg Dose Leads to Durable Responses in Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma. *Clinical cancer research : an official journal of the American Association for Cancer Research*, 23(5), 1149–1155. <https://doi.org/10.1158/1078-0432.CCR-16-1431>
- [50] Burger, J. A., Tedeschi, A., Barr, P. M., Robak, T., Owen, C., Ghia, P., Bairey, O., Hillmen, P., Bartlett, N. L., Li, J., Simpson, D., Grosicki, S., Devereux, S., McCarthy, H., Coutre, S., Quach, H., Gaidano, G., Maslyak, Z., Stevens, D. A., Janssens, A., ... RESONATE-2 Investigators (2015). Ibrutinib as Initial Therapy for Patients with Chronic Lymphocytic Leukemia. *The New England journal of medicine*, 373(25), 2425–2437. <https://doi.org/10.1056/NEJMoa1509388>

- [51] Dimopoulos, M. A., Tedeschi, A., Trotman, J., García-Sanz, R., Macdonald, D., Leblond, V., Mahe, B., Herbaux, C., Tam, C., Orsucci, L., Palomba, M. L., Matous, J. V., Shustik, C., Kastiris, E., Treon, S. P., Li, J., Salman, Z., Graef, T., Buske, C., & iNOVATE Study Group and the European Consortium for Waldenström's Macroglobulinemia (2018). Phase 3 Trial of Ibrutinib plus Rituximab in Waldenström's Macroglobulinemia. *The New England journal of medicine*, 378(25), 2399–2410. <https://doi.org/10.1056/NEJMoa1802917>
- [52] Burger J. A. (2019). Bruton Tyrosine Kinase Inhibitors: Present and Future. *Cancer journal* (Sudbury, Mass.), 25(6), 386–393. <https://doi.org/10.1097/PPO.0000000000000412>
- [53] Tam, C. S., Trotman, J., Opat, S., Burger, J. A., Cull, G., Gottlieb, D., Harrup, R., Johnston, P. B., Marlton, P., Munoz, J., Seymour, J. F., Simpson, D., Tedeschi, A., Elstrom, R., Yu, Y., Tang, Z., Han, L., Huang, J., Novotny, W., Wang, L., ... Roberts, A. W. (2019). Phase 1 study of the selective BTK inhibitor zanubrutinib in B-cell malignancies and safety and efficacy evaluation in CLL. *Blood*, 134(11), 851–859. <https://doi.org/10.1182/blood.2019001160>