Acalabrutinib (ACP-196): a selective second-generation BTK inhibitor

Jingjing Wu, Mingzhi Zhang and Delong Liu*

Abstract

More and more targeted agents become available for B cell malignancies with increasing precision and potency. The first-in-class Bruton’s tyrosine kinase (BTK) inhibitor, ibrutinib, has been in clinical use for the treatment of chronic lymphocytic leukemia, mantle cell lymphoma, and Waldenstrom’s macroglobulinemia. More selective BTK inhibitors (ACP-196, ONO/GS-4059, BGB-3111, CC-292) are being explored. Acalabrutinib (ACP-196) is a novel irreversible second-generation BTK inhibitor that was shown to be more potent and selective than ibrutinib. This review summarized the preclinical research and clinical data of acalabrutinib.

Background

New CD20 monoclonal antibodies and bispecific antibodies are providing more treatment options with increasing precision and potency for hematological malignancies [1–7]. Even though chronic lymphocytic leukemia (CLL) remains incurable at this time, more and more treatment options are bringing clinical benefits to patients with longer duration of response and less toxicities commonly seen with conventional chemotherapeutic agents [1, 8–11]. Bruton’s tyrosine kinase (BTK) is an essential kinase in the B cell receptor (BCR) signaling pathway and a driving force for CLL and other B cell malignancies [12–14]. The first-in-class BTK inhibitor, ibrutinib, has been in clinical use for the treatment of chronic lymphocytic leukemia (CLL), mantle cell lymphoma, and Waldenstrom’s macroglobulinemia [11, 13, 15–17]. However, ibrutinib has untoward effects, such as bleeding, rash, and atrial fibrillation, which could be partly due to the bystander effects on targets other than BTK [10, 13, 15, 17, 18]. Therefore, more selective BTK inhibitors (ACP-196, ONO/GS-4059, BGB-3111, CC-292) are being explored [19–23]. Acalabrutinib, also known as ACP-196, is a novel irreversible second-generation BTK inhibitor that was rationally designed to be more potent and selective than ibrutinib [19, 24–28]. This review summarized the preclinical research and clinical data of acalabrutinib.

Mechanism of action and properties of acalabrutinib

Acalabrutinib binds covalently to Cys481 with improved selectivity and in vivo target coverage compared to ibrutinib and CC-292 in CLL patients [19, 20, 26]. In the in vitro signaling assay on primary human CLL cells, acalabrutinib inhibited tyrosine phosphorylation of downstream targets of ERK, IKB, and AKT [24]. Acalabrutinib demonstrated higher selectivity for BTK with IC50 determinations on nine kinases with a cysteine residue in the same position as BTK [19]. Importantly, unlike ibrutinib, acalabrutinib did not inhibit EGFR, ITK, or TEC [19, 24]. In the in vitro assays reported in the supplemental data, it was clearly demonstrated that, unlike ibrutinib, acalabrutinib had no effect on EGFR phosphorylation on tyrosine residues y1068 and y1173. At 1000 nM, ibrutinib completely suppressed Tec activity, though 1000 nM acalabrutinib had minimal activity on Tec [24]. Compared with ibrutinib, acalabrutinib has much higher IC50 (>1000 nM) or virtually no inhibition on kinase activities of ITK, ERBB2, ERBB4, JAK3, BLK, FGR, FYN, HCK, LCK, LYN, SRC, and YES1 [24].

The differential effects of acalabrutinib on primary CLL cells, T cells, NK cells, and epithelial cells were studied by signaling and functional assays. Acalabrutinib inhibited purified BTK with an IC50 of 3 nM and EC50 of 8 nM in a human whole-blood CD69 B cell activation assay [19]. Acalabrutinib was shown to have improved target specificity over ibrutinib with 323-, 94-, 19-, and 9-fold selectivity over the other TEC kinase family members (ITK, TXK, BMX, and TEC, respectively) and no activity against EGFR.
The effects of ibrutinib and acalabrutinib on platelets were also compared in an in vivo VWFHA1 mouse thrombosis model. The platelets from patients treated with ibrutinib 420 mg once per day or acalabrutinib 100 mg twice per day were assessed for thrombus formation at injured arterioles of the mice. The thrombus sizes from acalabrutinib-treated platelets were comparable to those of healthy controls, whereas thrombus formation was clearly inhibited in ibrutinib-treated platelets. These data suggest that acalabrutinib, unlike ibrutinib, is more selective for inhibiting BTK and has virtually no inhibition of platelet activity [24].

There data clearly suggest that acalabrutinib is a more selective and potent second-generation BTK inhibitor.

**Acalabrutinib (ACP-196) in preclinical research**

Acalabrutinib was evaluated in several animal models of B cell non-Hodgkin lymphoma (NHL). These studies provided preclinical in vivo data necessary to move acalabrutinib into human trials. In a study of canine model of B cell NHL, 12 dogs with B cell NHL were orally administered acalabrutinib at escalating dosages of 2.5 mg/kg every 24 h (6 dogs), 5 mg/kg every 24 h (5 dogs), or 10 mg/kg every 12 h (1 dog). As a result, 3 dogs achieved a partial remission (PR), 3 dogs had stable disease (SD), whereas the remaining 6 dogs had progression of disease (PD). This study therefore showed that acalabrutinib has single agent biologic activity in a spontaneous large animal model of NHL [25].

The in vivo effects of acalabrutinib against CLL cells were demonstrated in the NSG mouse model with xenografts of human CLL [28]. Acalabrutinib significantly inhibited proliferation of human CLL cells in the spleens of NSG mice at all dose levels, as measured for the expression of Ki67 ($P = 0.002$). Tumor burden decreased with the treatment of acalabrutinib in a dose-dependent manner. Acalabrutinib inhibited BCR signaling by reduced phosphorylation of PLCγ2. Acalabrutinib transiently increased CLL cell counts in the peripheral blood. Therefore, the novel BTK inhibitor acalabrutinib shows in vivo efficacy against human CLL cells xenografted to the NSG mouse model.

Two murine models were used in another in vivo study [29, 30]. In the TCL1 adoptive transfer model, acalabrutinib inhibited BCR signaling by decreased autophosphorylation of BTK and reduction in surface expression of the BCR activation markers CD86 and CD69. Most interestingly, acalabrutinib treatment increased survival significantly over mice receiving vehicle (median 81 vs 59 days, $P = 0.02$). The second murine model was the NSG xenograft model. Acalabrutinib treatment significantly decreased the phosphorylation of PLCγ2 and ERK ($P = 0.02$), reduced tumor cell proliferation ($P = 0.02$), and tumor burden ($P = 0.04$).

**Table 1** Acalabrutinib (ACP-196) for hematological malignancies

| Agents                          | Diseases                                      | Phase   | NCT      |
|--------------------------------|-----------------------------------------------|---------|----------|
| ACP-196                         | MM                                            | Phase 1 | NCT02211014 |
| Dexamethasone                   | ABC DLBCL                                     | Phase 1 | NCT02112526 |
| ACP-196                         | FL                                            | Phase 1 | NCT02180711 |
| Rituximab (IV)                  | CLL, SLL, PLL                                 | Phase 1 | NCT02296918 |
| ACP-196                         | CLL                                           | Phase 1 | NCT02157324 |
| Obinutuzumab                    | NHL, MM, B-ALL                                | Phase 1/2 | NCT02328014 |
| ACP-196                         | WM                                            | Phase 1/2 | NCT02180724 |
| ACP-319                         | CLL                                           | Phase 1/2 | NCT02029443 |
| ACP-196                         | NHL, MM, H, CLL, RS, WS, WM, Myelofibrosis    | Phase 1/2 | NCT02362035 |
| ACP-196                         | CLL                                           | Phase 2 | NCT02337829 |
| ACP-196                         | MCL                                           | Phase 2 | NCT02213926 |
| ACP-196                         | CLL                                           | Phase 3 | NCT02475681 |
| Obinutuzumab Chlorambucil       | CLL                                           | Phase 3 | NCT02477696 |

NHL non-Hodgkin lymphoma, HL Hodgkin lymphoma, MM multiple myeloma, MCL mantle cell lymphoma, FL follicular lymphoma, ABC DLBCL activated B cell diffuse large B cell lymphoma, CLL chronic lymphocytic leukemia, SLL small lymphocytic lymphoma, WM Waldenstrom’s macroglobulinemia, RS Richter’s syndrome, PLL prolymphocytic leukemia
Acalabrutinib was shown to be a potent inhibitor of BTK in both murine models of human CLL [29].

**Acalabrutinib in clinical development**

To further assess the safety, efficacy, pharmacokinetics, and pharmacodynamics of acalabrutinib in human CLL, a phase 1/2, multicenter, open-label, and dose-escalation clinical trial has been ongoing (NCT02029443). In the latest report, 61 patients with relapsed CLL were enrolled [24]. These patients had received a median of three prior therapies for CLL. Among them, 31 % were positive for 17p13.1 deletion and 75 % had IgVH unmutated. In the phase 1 portion of this study, patients were treated with acalabrutinib at an increasing dose of 100 to 400 mg once daily. In the phase 2 expansion portion, 100 mg twice daily was given. After a median follow-up of 14.3 months (range 0.5–20), the overall response rate (ORR) was 95 %, with 85 % PR, 10 % PR with lymphocytosis. The remaining 5 % of patients were reported to have SD. The ORR was 100 % in those patients with chromosome 17p13.1 deletion. Headache, diarrhea, and weight gain were the most common adverse events. There were no dose-limiting toxicities observed in the phase 1 portion of the trial. There was no Richter’s transformation and no cases of atrial fibrillation have been reported to date [24]. The preclinical data from this report confirmed that acalabrutinib is a highly selective BTK inhibitor. It does not inhibit TEC kinase and platelet aggregation which would be a preferred advantage over ibrutinib and would have reduced bleeding risk. Acalabrutinib does not inhibit EGFR, thus could reduce the adverse events on skin rash and severe diarrhea. Transient headaches were reported to be a common adverse event from acalabrutinib and appeared to be more frequently seen than in those patients on ibrutinib from historical data. More patients and longer follow-up from this study are needed to ascertain these potential advantages and disadvantages. In addition, direct comparison of acalabrutinib with ibrutinib will be the only way to confirm the advantages and disadvantages of these agents.

At this time, a phase 3 study (NCT02477696) has commenced in which acalabrutinib is being compared with ibrutinib in high-risk patients with relapsed CLL. Studies in treatment-naive CLL are also being done (Table 1).

**Conclusion and future directions**

Acalabrutinib is a more selective irreversible second-generation BTK inhibitor. It has improved target specificity and enhanced potency for BTK due to reduced off-target activity on EGFR, TEC, etc., which may lead to less untoward effects and toxicities. More patients and longer follow-up from the ongoing phase I/II clinical study are needed to ascertain these potential advantages. Multiple trials on other hematological malignancies and solid tumors are underway (Tables 1 and 2). Combination of acalabrutinib and other agents (new CD19 and CD20 antibodies, BCL-2 inhibitors, PI3 kinase inhibitors, ALK inhibitors, etc.) for B cell malignancies will likely further increase response rate and duration of response [1, 8, 31–37] (Table 1). It will also be interesting to see whether resistance mechanisms will be different from those for ibrutinib. Finally, additional selective BTK inhibitors, i.e., ONO/GS-4059 and BGB-3111, are under active clinical development [21–23]. More options of treatment may become available for B cell malignancies.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

DL designed the study. JW and DL drafted the manuscript. MZ participated in the manuscript preparation and revisions. All authors read and approved final manuscript.

---

**Table 2** Acalabrutinib (ACP-196) for solid tumors

| Agents | Diseases | Phase | NCT  |
|--------|----------|-------|------|
| ACP-196 | Glioblastoma multiforme | Phase 1/2 | NCT02586857 |
| ACP-196 Pembrolizumab | Urothelial carcinoma | Phase 2 | NCT02351739 |
| ACP-196 Pembrolizumab | Non-small-cell lung cancer | Phase 2 | NCT02448303 |
| ACP-196 Pembrolizumab | Head and neck cancer | Phase 2 | NCT02454179 |
| ACP-196 Pembrolizumab | Squamous cell carcinoma | Phase 2 | NCT02537444 |
| ACP-196 Pembrolizumab | Ovarian cancer | Phase 2 | NCT02362048 |
| ACP-196 Pembrolizumab | Pancreatic cancer | Phase 2 | NCT02570711 |
Acknowledgement

Jingjing Wu is a recipient of the Henan Provincial Grant for Overseas Research for Young Leaders of Medical Technology (No. 2014041). She also received grant support from the Natural Science Foundation of China (NSFC No. 81201793). The grants supported her research training at the Division of Hematology and Oncology, New York Medical College and Westchester Medical Center, Valhalla, NY, USA.

Received: 23 February 2016 Accepted: 1 March 2016
Published online: 09 March 2016

References

1. Goede V, Fischer K, Busch R, Engelke A, Eichhorst B, Wendtner CM, et al. Obinutuzumab plus chlorambucil in patients with CLL and coexisting conditions. N Engl J Med. 2014;370(12):101–10.
2. Fan G, Wang Z, Hao M, Li J. Bispecific antibodies and their applications. J Hematol Oncol. 2015;8(1):1–14.
3. Reusch U, Duell J, Ellwanger K, Herbrecht C, Knackmuss SH, Fucek I, et al. A tetravalent bispecific TandAb (CD19/CD3), AFM13, efficiently recruits T cells for the potent lysis of CD19+ tumor cells. MAbs. 2015;7(5):584–604.
4. Suresh T, Lee L, Joshi J, Barta S. New antibody approaches to lymphoma therapy. J Hematol Oncol. 2014;7(1):58.
5. Thomas X. Blinatumomab: a new era of treatment for adult ALL? Lancet Oncol. 2014;16(16):16–17.
6. Wu J, Fu J, Zhang M, Liu D. AFM13: a first-in-class tetravalent bispecific anti-CD30/CD16A antibody for NK cell-mediated immunotherapy. J Hematol Oncol. 2015;8:96.
7. Wu J, Fu J, Zhang M, Liu D. Blinatumomab: a bispecific T cell engager (BiTE) antibody against CD19/CD3 for refractory acute lymphoid leukemia. J Hematol Oncol. 2015;8(1):104.
8. Cang S, Iragavarapu C, Savoji J, Song Y, Liu D. ABT-199 (venetoclax) and BCL-2 inhibitors in clinical development. J Hematol Oncol. 2015;8(1):123.
9. Roberts AW, Davids MS, Pagel JM, Kahl BS, Puvvada SD, Green R, et al. Targeting BCL2 with venetoclax in relapsed chronic lymphocytic leukemia. N Engl J Med. 2016;374(4):311–22.
10. Rai K. Therapeutic potential of new B cell-targeted agents in the treatment of elderly and unfit patients with chronic lymphocytic leukemia. J Hematol Oncol. 2015;8(1):85.
11. Novero A, Ravella PM, Chen Y, Dous G, Liu D. Ibrutinib for B cell malignancies. Experimental Hematology & Oncology. 2014;3(1):1–7.
12. Treon SP, Xu L, Hunter Z. MYD88 mutations and response to ibrutinib in Waldenstrom’s macroglobulinemia. N Engl J Med. 2015;373(6):584–6.
13. Treon SP, Tripsas CK, Meid K, Warren D, Varma G, Green R, et al. Ibrutinib in previously treated Waldenstrom’s macroglobulinemia. N Engl J Med. 2015;372(1):430–40.
14. Cao Y, Yang G, Hunter ZR, Liu X, Xu L, Chen J, et al. The BCL2 antagonist ABT-199 triggers apoptosis, and augments ibrutinib and ibrutinib-mediated cytotoxicity in CCR45 wild-type and CCR45 WHIM mutated Waldenstrom macroglobulinemia cells. Br J Haematol. 2015;170(1):134–8.
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(1):57–16.
16. Braun JP, Barrientos JC, Barr PM, Flinn IW, Burger JA, Tran A, et al. The Bruton tyrosine kinase inhibitor ibrutinib with chemoimmunotherapy in patients with chronic lymphocytic leukemia. Blood. 2015;125(19):2915–22.
17. Burger JA, Tedeschi A, Barr PM, Robak T, Owen C, Gha P, et al. Ibrutinib as initial therapy for patients with chronic lymphocytic leukemia. N Engl J Med. 2015;373(25):2425–37.
18. Cang S, Cang S, Sekhi A, Liu D. Selective inhibitors of nuclear export (SINE) in hematological malignancies. Exp Hematol. 2015;43(7):1537–42.
19. Niemann CU, Montraveta A, Herman SEM, Ingallinera T, Barf T, Colomer D, et al. Abstract 2624: the novel Bruton’s tyrosine kinase inhibitor ACP-196 shows in vivo efficacy against human chronic lymphocytic leukemia cells xenografted to the NSG mouse model. Cancer Res. 2014;74(19 Supplement):12624.
20. Herman SEM, Montraveta A, Niemann CU, Mora-Jensen H, Gulrajani M, Kozak E, et al. The Bruton tyrosine kinase (BTK) inhibitor ACP-196 demonstrates clinical activity in two mouse models of chronic lymphocytic leukemia. Blood. 2015;126(23):2920.
21. Byrd JC, Furman RR, Coutre SE, Flinn IW, Burger JA, Tran A, et al. The Bruton’s tyrosine kinase inhibitor ibrutinib is safe, tolerable, and highly active in patients with relapsed/refractory B-cell malignancies: initial report of a phase 1 first-in-human trial. Blood. 2015;126(23):832.
22. Tam C, Grigg AP, Opat S, Ku M, Gilbertson M, Anderson MA, et al. The BTK inhibitor, BGb-3111, is safe, tolerable, and highly active in patients with relapsed/refractory B-cell malignancies: initial report of a phase 1 first-in-human trial. Blood. 2015;126(23):832.
23. Walter HS, Rule SA, Dyer MJ, Karlin L, Jones C, Cazin B, et al. A phase 1 clinical trial of the selective BTK inhibitor ONO/GS-4059 in relapsed and refractory B cell malignancies. Blood. 2016;127(4):411–9.
24. Byrd JC, Harrington B, O’Brien S, Jones JA, Schuh A, Devereaux S, et al. Acalabrutinib (ACP-196) in relapsed chronic lymphocytic leukemia. N Engl J Med. 2016;374(4):323–32.
25. Gardner HL, Harrington* BK, Irmay R, Hamdy A, Kaptein A, Lith BV, et al. Abstract 1744: ACP-196: a second generation Btk inhibitor demonstrates biologic activity in a canine model of B-cell non-Hodgkin lymphoma. Cancer Res. 2014;74(19 Supplement):1744.
26. Harrington BK, Gulrajani M, Covey T, Kaptein A, Van Lith B, Izumi R, et al. ACP-196 is a second generation inhibitor of Bruton tyrosine kinase (BTK) with enhanced target specificity. Blood. 2015;126(23):2908.
27. Lannutti BJ, Gulrajani M, Kozak E, Bibikova E, Covey T, Jenson K, et al. Abstract 408: ACP-196, an orally bioavailable covalent selective inhibitor of Btk, modulates the innate tumor microenvironment, exhibits antitumor efficacy and enhances gemcitabine activity in pancreatic cancer. Cancer Res. 2015;75(15 Supplement):408.
28. Niemann CU, Montraveta A, Herman SEM, Ingallinera T, Barf T, Colomer D, et al. Abstract 2624: the novel Bruton’s tyrosine kinase inhibitor ACP-196 shows in vivo efficacy against human chronic lymphocytic leukemia cells xenografted to the NSG mouse model. Cancer Res. 2014;74(19 Supplement):12624.
29. Herman SEM, Montraveta A, Niemann CU, Mora-Jensen H, Gulrajani M, Kozak E, et al. The Bruton tyrosine kinase (BTK) inhibitor ACP-196 demonstrates clinical activity in two mouse models of chronic lymphocytic leukemia. Blood. 2015;126(23):2920.
30. Herman SEM, Montraveta A, Niemann CU, Mora-Jensen H, Gulrajani M, Kozak E, et al. The Bruton’s tyrosine kinase inhibitor ACP-196 demonstrates clinical activity in two mouse models of chronic lymphocytic leukemia. Blood. 2015;126(23):2920.
31. Rai KR. Barrientos JC. Movement toward optimization of CLL therapy. N Engl J Med. 2014;370(12):160–2.
32. Yang Q, Modr P, Newcomb T, Queva C, Gandhi V. Idealisib: first-in-class PI3K delta inhibitor for the treatment of chronic lymphocytic leukemia, small lymphocytic leukemia, and follicular lymphoma. Clin Cancer Res. 2015;21(7):1537–42.
33. Iragavarapu C, Mustafa M, Akinleye A, Furqan M, Mittal V, Cang S, et al. Novel ALK inhibitors in clinical use and development. J Hematol Oncol. 2015;8(1):17.
34. Das A, Wei G, Parikh K, Liu D. Selective inhibitors of nuclear export (SINE) in hematological malignancies. Exp Hematol. 2015;43(7):1537–42.
35. 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 efficacy while sparing platelets. Nat Med. 2013;19(2):202–8.
36. Zhao X, Rajasekaran N, Reusch U, Weichel M, Ellwanger K, Marschner J-P, et al. In vitro and in vivo characterization of CD19/CD3 Tandab AFF11 and CD19/CD16A Tandab AFF12 targeting NHL. Blood. 2015;126(23):2763.