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Chronic Myeloid Leukemia: Advances in Understanding Disease Biology and Mechanisms of Resistance to Tyrosine Kinase Inhibitors

Christopher A. Eide, BA[1,2] and **Thomas O'Hare, PhD**[3,4]

[1]Division of Hematology and Medical Oncology, Oregon Health & Science University Knight Cancer Institute, Portland, OR, USA

[2]Howard Hughes Medical Institute, Portland, OR, USA

[3]Huntsman Cancer Institute, University of Utah, Salt Lake City, UT, USA

[4]Division of Hematology and Hematologic Malignancies, University of Utah, Salt Lake City, UT, USA

Abstract

The successful implementation of tyrosine kinase inhibitors (TKIs) for the treatment of chronic myeloid leukemia (CML) remains a flagship for molecularly targeted therapy in cancer. This focused review highlights critical elements of the underlying biology of CML and provides a summary of the molecular mechanisms that lead to TKI resistance: BCR-ABL1 mutation-based resistance and therapy escape through alternative pathway activation despite inhibition of BCR-ABL1 tyrosine kinase activity. We direct attention to the most current manifestations of these issues, including emergence of pan-TKI-resistant BCR-ABL1 compound mutants, new strategies for identification and therapeutic targeting of alternative pathways, and the exciting, controversial topic of cessation of TKI therapy leading to durable treatment-free remissions for a subset of patients. Further gains in our understanding of the biology of Philadelphia chromosome-positive (Ph-positive) leukemia and mechanisms of resistance to BCR-ABL1 TKIs will benefit patients and also provide a blueprint for similar discovery in other cancers.

Keywords

Chronic myeloid leukemia (CML); tyrosine kinase inhibitor (TKI); BCR-ABL1 compound mutation; treatment-free remission (TFR); BCR-ABL1-independent resistance; imatinib

Correspondence: Thomas O'Hare, 2000 Circle of Hope, Room 4264, Salt Lake City, UT 84112, USA: Ph: 801-585-0256; Thomas.OHare@hci.utah.edu.

Conflict of Interest

Dr. Christopher A. Eide declares that he has no conflict of interest. Dr. Thomas O'Hare declares that he has no conflict of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

Introduction – Tyrosine Kinase Inhibitors in Chronic Myeloid Leukemia

The paradigm of tyrosine kinase inhibitors (TKIs) for the treatment of patients with chronic myeloid leukemia (CML) is a success story of the highest order in molecularly targeted therapy [1, 2]. The majority of chronic phase CML patients treated with first-line imatinib demonstrate rapid achievement of durable remission, and five-year overall and progression-free survival rates approach 90%. For those patients who fail therapy due to resistance or intolerance, a quiver of additional FDA-approved second-generation TKIs are available for deployment. In total, five TKIs are approved for first-line and/or salvage treatment of CML in the U.S.: imatinib, dasatinib, nilotinib, bosutinib, and ponatinib (Table 1). The recent approval of nilotinib [3] and dasatinib [4] for first-line CML therapy, along with the ongoing clinical evaluation of bosutinib for first-line and salvage use [5–7] and the approval of the pan-BCR-ABL1 inhibitor ponatinib for refractory CML [8], has also broadened prospects for minimizing resistance and maximizing long-term disease control.

These clinical advances have largely capitalized on the molecular biology of CML, wherein the BCR-ABL1 kinase encoded by the t(9;22) chromosomal translocation is importantly present in all CML cells but not normal cells and required for disease transformation [9–11]. The fusion of BCR and ABL1 facilitates dimerization of BCR-ABL1 proteins via the coiled-coil domain of BCR. Mutual transphosphorylation of the juxtaposed ABL1 kinase domains results in constitutively active tyrosine kinase activity. Activated BCR-ABL1 drives a variety of downstream pro-survival, growth, and anti-apoptosis signaling pathways including JAK/STAT, RAS/RAF/MEK/ERK, PI3K/AKT, and BAD/BCL-X_L. Furthermore, expression of BCR-ABL1 in murine bone marrow transplantation models results in an aggressive myeloid leukemia phenotype that remains sensitive to treatment with ABL1 TKIs [12].

If left untreated clinically, CML progresses from a chronic phase (CP-CML) featuring excessive proliferation of the full lineage of myeloid cells in the bone marrow through an accelerated phase to an unstable and aggressive blast crisis (which may present in myeloid or lymphoid form) characterized by excessive marrow and peripheral blood blasts and a block in differentiation. Additionally, approximately 20–30% of adult cases and 3–5% of pediatric cases of Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph-positive ALL) harbor the t(9;22) translocation. TKIs targeting BCR-ABL1 are often also part of the treatment approach for these patients, but in this setting and in the advanced phases of CML, responses are almost inevitably transient and the prognosis remains poor compared to CP-CML.

Given the significant milestones achieved in understanding the molecular pathogenesis of CML and the successful implementation of TKIs in its treatment to date, there may be a perception that "CML is done" as far as the need for additional research. While we wish this were true, several challenging hurdles remain to be cleared. Here, we survey and discuss progress on three of the most pressing current problems pertaining to the treatment and management of in Ph-positive leukemia.

Problem 1: Emergence of TKI-resistant versions of BCR-ABL1

In the field of TKI resistance, the oldest trick in the book is acquisition of a mutation in the gene encoding the target enzyme that results in decreased or eliminated inhibitor effectiveness without undue compromise in catalytic function. In the case of CML, approximately 20–30% of patients initially treated with imatinib will develop resistance to therapy, most commonly through gain of point mutations within the kinase domain of BCR-ABL1 that directly interfere with or indirectly disfavor imatinib binding. To date, over 100 different BCR-ABL1 kinase domain mutations have been reported in resistance to imatinib and to a lesser extent, second-generation TKIs, primarily centered around or within the phosphate binding loop (P-loop), ATP binding site, and activation loop [2, 13, 14].

With respect to single mutations in BCR-ABL1, this clinical challenge has been met with a succession of second-generation TKIs (Table 1). In principle, there is now a therapeutic TKI option for every clinically reported BCR-ABL1 point mutant linked to TKI resistance [2]. In the case of the gatekeeper T315I mutant, the only TKI approved for clinical use is ponatinib [15]. Despite the clinical availability of five TKIs, when one considers the limited options for certain mutations, most notably BCR-ABL1^{T315I}, as well as patient-to-patient variability in pharmacokinetic and TKI tolerability profiles, it is our opinion that there is still a small, unmet need in the area of TKIs for BCR-ABL1 point mutations.

A so-far rare but much more concerning unmet need is a strategy to deal with BCR-ABL1 compound mutants, in which the identities of two or more amino acid residues are changed in the same BCR-ABL1 molecule (Fig. 1) [16]. Generally thought to emerge under selective pressure associated with sequential TKI treatment, over 60 different BCR-ABL1 compound mutations have been reported to date in connection with TKI resistance [16–21]. Evidence that BCR-ABL1 kinase has limited tolerance to accrue successive missense mutations indicates that TKIs with activity against compound mutants may block mutational escape [17, 22]. Drug sensitivity profiling of a broad panel of two-component compound mutants indicates that T315I-inclusive compound mutants in particular confer a high degree of resistance to all currently available BCR-ABL1 TKIs, suggesting that patients who harbor such a mutation may have very limited therapeutic options and underscoring the need to distinguish polyclonal mutations from true compound mutations [16–18]. Furthermore, many patients who start on ponatinib therapy harbor a T315I mutation at baseline and T315I-inclusive compound mutations have been confirmed in a subset of clinical failures of ponatinib [8, 18]. This warrants close monitoring of such patients for evidence of potential emergence of compound mutants. By contrast, BCR-ABL1 compound mutants that do not include a T315I component show variable sensitivity to the clinically available TKIs, such that one or more TKI may represent a rational treatment option [18]. Additionally, we reported that a small group of key positions is highly represented in clinically observed two-component compound mutants, with select positions (e.g. 315) pairing with nearly all other key positions and some positions (e.g. 252) only pairing with one or two others [18]. Although early detection of extremely low-level BCR-ABL1 compound mutations may eventually prove to be of diagnostic value, technical limitations dating back to the first reports on BCR-ABL1 compound mutations remain to be overcome [16, 17, 23].

An intriguing possibility for sidestepping the problem of BCR-ABL1 kinase domain mutation-mediated TKI resistance in CML is to disable BCR-ABL1 using an allosteric inhibitor directed to a site other than the ATP pocket. A series of studies with the related compounds, GNF-2 and GNF-5, validated this concept. Somewhat surprisingly, perhaps, is the reported observation that engagement of these allosteric inhibitors by BCR-ABL1 restored the efficacy of BCR-ABL1 TKIs, including nilotinib against the T315I mutant [24–27]. More recently, Novartis has initiated a phase 1, multicenter, open-label study of oral ABL001, a BCR-ABL1 myristate site binder that enforces an autoinhibited conformation of the enzyme, in patients with refractory CML or Ph-positive ALL (http://clinicaltrials.gov/show/NCT02081378; Table 1). Capacity of such inhibitors to block compound mutations as well as the possibility of acquired resistance mutations within the allosteric binding site emerging clinically will require additional investigation.

While a subset of BCR-ABL1 compound mutations represent a formidable clinical challenge at present, longer clinical experience and establishment of a universal, gold standard method that can accurately detect low level compound mutations may permit determination of whether a limited set of compound mutants account for most instances of therapy escape via this route. Efforts to understand the scope of this problem and to develop a structural rationale for targeting compound mutants are ongoing.

Problem 2: Some patients experience treatment failure despite effective BCR-ABL1 inhibition

From the earliest reports of clinical resistance to TKI therapy in CML, it has been clear that BCR-ABL1 mutations are not always the explanation for relapse. Now that the field has become reasonably proficient at controlling BCR-ABL1 mutation-based resistance, the quest to understand and intercept other escape routes has finally begun to get the attention it requires. In BCR-ABL1-independent resistance, TKIs successfully suppress BCR-ABL1 kinase activity, but alternative signaling through pathways such as SRC [28, 29], PI3K [30], KRAS [31] and JAK2 [32] compensate for this loss (Fig. 1B). However, no unifying concept of BCR-ABL1-independent resistance has emerged and clinical management relies on cytotoxic agents or transplant.

The inability to identify and target BCR-ABL1-independent alternative pathways has important clinical consequences. Interim results from a phase 1 study of ponatinib in patients with refractory Ph-positive leukemia in which 94% of enrolled patients had failed ≥2 prior TKIs demonstrated that responses occurred irrespective of BCR-ABL1 mutation status and were mostly durable in CP-CML, but major molecular response rates were lower in patients without evidence of a BCR-ABL1 mutation at baseline, suggesting involvement of BCR-ABL1-independent mechanisms in ponatinib resistance [33]. Further analysis from the large, ongoing phase 2 study (PACE trial) suggests that at least half of the occurrences of clinical ponatinib resistance cannot be explained by BCR-ABL1 single or compound mutations, similarly implicating activation of alternative co-critical pathways. In addition, given that responses to any of the clinically available BCR-ABL1 TKIs are generally poorer and transient rather than durable in patients with advanced CML or Ph-positive ALL, and it is unlikely that any single agent TKI, including ponatinib, will change this. To contend with

the range of resistance mechanisms, we need TKIs that target compound mutants and we also need to clinically implement TKI/second inhibitor combinations that induce synthetic lethality.

Recent studies on NFAT [34] and MEK [35–37] feedback signaling circuits, for example, reveal new targets for synthetic lethality approaches in therapy-resistant CML and for targeting CML stem cells. Ma and colleagues recently employed a large-scale shRNA-based screen to identify a subset of genes whose down-regulation resulted in decreased imatinib sensitivity in CML cells [37]. While the individual genes implicated were quite varied, nearly all conditions exhibited persistent RAF/MEK/ERK signaling activity attributed to increased PRKCH expression despite inhibition of BCR-ABL1 kinase activity by TKIs. Intriguingly, the combination of BCR-ABL1 TKIs with the MEK inhibitor trametinib resulted in synergistic kill of these cells and prolonged survival in a CML mouse model of BCR-ABL1-independent resistance. Notably, the concept of synthetic lethality opportunity between BCR-ABL1 and MEK targets is also in line with previous studies describing paradoxical RAS-dependent activation of RAF/MEK/ERK in nilotinib-treated CML cells harboring drug-resistant BCR-ABL1 mutations [35] and the role of high levels of MEK-dependent negative feedback in BCR-ABL1-mediated oncogene addiction [36].

Another strategy that has begun to be explored rather than trying to identify and inhibit individual, proximal signaling pathways is to target TKI-induced feedback activation of STAT3 [38, 39]. STAT3 is a mediator of extrinsic TKI resistance conferred on CML cells by bone marrow-derived factors [40, 41]. $STAT3^{Y705}$ is phosphorylated in TKI-resistant primary CML cells in a cell-autonomous (intrinsic) fashion, suggesting that $pSTAT3^{Y705}$ integrates intrinsic and extrinsic resistance pathways. As leukemia cells have limited ways to compensate for loss of BCR-ABL1 signaling, a signal integrator such as STAT3 is perhaps an ideal therapeutic target. Targeting transcription factors is notoriously difficult, and development of BP-5-087, a novel and potent mechanism-based STAT3 inhibitor, required a choreographed combination of synthetic chemistry, sensitive in vitro reporter assays and dynamic computational modeling. Additional structure-activity relationship and absorption, distribution, metabolism, excretion studies are ongoing, with the goal of bringing clinical STAT3 inhibitors within reach for the first time [38]. As the field delves further into exploitable resistance mechanisms for novel therapeutic intervention, it is our viewpoint that findings with respect to effective synthetic lethality approaches in Ph-positive leukemia will inform similar strategies in other malignancies such as acute myeloid leukemia (AML).

Along with more thoroughly defining mechanisms of resistance, considerable progress has been made in understanding the additional complexities that define advanced CML and Ph-positive ALL as compared to CP-CML. For example, Beer and colleagues reported that protein levels of the tumor suppressor IKAROS are barely detectable or absent in bone marrow blasts in the majority of CML patients with advanced myeloid disease, compared with substantial levels in CP-CML cells [42]. Forced expression of IK6, a dominant negative isoform of IKAROS, in $CD34^{+}$ CP-CML cells *in vitro* conferred features of accelerated phase CML. Deletion of *IKAROS* has also been previously reported in Ph-positive ALL [43]. These findings link loss or reduction of IKAROS to advanced as compared to chronic phase disease, providing a potential biomarker for impending disease progression.

Problem 3: TKI therapy is not curative; most patients require lifelong TKI therapy

Even at the level of phase 1 clinical trials, imatinib demonstrated astounding efficacy. In the ensuing 15 years, the practice of TKI-based disease management has been continuously improved. One point, however, has always been taken as gospel: TKIs enforce maximum disease control but do not target stem cells and are not curative. As such, any patient discontinuing TKI therapy would be expected to be at risk of immediate or eventual relapse, and there is substantial anecdotal clinical evidence and underlying CML stem cell biology supporting this assumption [44, 45]. This of course also has very significant implications for the financial burden of the treatment of the disease for patients.

The impetus to characterize and effectively target CML at its hematopoietic roots has been a long fought battle. CML originates in the hematopoietic stem cell compartment, and is renewed by poorly defined leukemic stem cells (LSCs). As best we can experimentally determine, LSCs are *BCR-ABL1*-positive, though whether they express high or low levels of BCR-ABL1 is controversial [46]. Our working hypothesis is that TKIs are, in principle, capable of reaching LSCs and blocking their BCR-ABL1 activity but that this intervention is insufficient to eliminate LSCs [47, 48]. In other words, LSCs are not solely or strictly dependent on BCR-ABL1 kinase activity for survival. There is also evidence that the bone marrow niche is a hypoxic microenvironment that may act to promote LSC maintenance independent of BCR-ABL1 kinase activity, suggesting that combining BCR-ABL1 TKIs with inhibitors of hypoxia-inducible factor 1 α signaling may be a feasible strategy for LSC eradication [49]. In recent reports, several factors have been convincingly implicated in CML LSC maintenance, survival, and resistance to TKI therapy, including arachidonate 15-lipoxygenase [50], the IL-2/CD25 signaling axis [51], and the Wnt/β-catenin axis as influenced by N-cadherin [52]. Furthermore, given that in vitro studies do not reflect all of the barriers inherent in the bone marrow microenvironment [53], recent evidence suggests blockade of adhesion molecule-ligand interactions that are more important for homing and engraftment of LSCs than normal HSCs is a strategy for improving accessibility of TKIs to these cells [54, 55].

Despite this somewhat engrained dogma of CML LSC persistence and inevitable relapse upon stopping treatment, however, some patients who achieved and sustained deep molecular responses for years on TKI therapy accepted the terms of a carefully conceived and controlled clinical trial to see if treatment-free remission (TFR) is possible, and the findings continue to be both intriguing and incompletely understood [56]. The first large-scale trial exploring this question was called STop IMatinib (STIM) (Fig. 1) [57]. A different set of investigators carried out the similarly designed TWISTER study, which utilized a slightly different trigger point for restarting TKI therapy [58]. The interim results for the two trials are remarkably congruent, with in the neighborhood of 40% of patients maintaining TFR at two years, and the vast majority of patients experiencing molecular recurrence doing so within the first seven months after treatment cessation.

Of note, this is ~40% of an already very select population characterized by deep, durable molecular response to TKIs as assessed by quantitative RT-PCR of *BCR-ABL1* transcript

levels indexed to an international scale [59]. All in all, only ~5% of patients are likely to be eligible for TKI cessation. Current efforts and trial designs are geared toward determining whether use of second-generation TKIs such as dasatinib [4] or nilotinib increase the rate of TFRs, either in the first-line setting or after suboptimal response on imatinib [60, 61]. There is also emphasis on defining the best threshold for trial enrollment and for mandating re-start of TKI therapy [62]. It is becoming clear that these values will need to be tailored to specific situations, as exemplified by the nilotinib-based ENESTcmr trial [61] and follow-up suite of TFR studies (ENESTfreedom, ENESTop, ENESTgoal, ENESTpath).

For the time being, the exciting and somewhat daring prospect of stopping TKI therapy and monitoring for TFR is panning out spectacularly for a small minority of patients [56], but we are not sure how to prospectively identify these patients [62]. One certainty is that any plan to test the waters of TFR at this time should be done only in the setting of a clinical trial. Extensive effort into determining TFR-specific signatures is of great interest and warrants the attention of the field.

Closing Thoughts and Outlook

Many of us will face cancer in our lifetime, and certainly none of us will view it as good news. For those who receive a diagnosis of CML, the availability of TKIs that target the enzymatic activity of the causative BCR-ABL1 fusion tyrosine kinase provides an effective treatment strategy but generally not a cure. Beginning with the regulatory approval of imatinib in May of 2001, the use of TKIs in CML has been honed to a fine art, much to patients' benefit.

Key current issues include the need for design and clinical implementation of TKIs that inhibit BCR-ABL1 compound mutants and development of inhibitor combinations targeting BCR-ABL1 and alternative pathways. TKI resistance in several other cancers also involves either compound mutations or alternative pathway activation, suggesting a general principle in kinase-targeted therapy. For example, FLT3 ITD-positive AML patients resistant to quizartinib (AC220) exhibit secondary mutations in the kinase activation loop, a subset of which are ponatinib-sensitive [63–65]. Many gastrointestinal stromal tumor (GIST) patients with resistance to imatinib and sunitinib exhibit compound mutations including the KIT gatekeeper residue; overexpression of AXL or focal adhesion kinase is implicated in some cases without secondary KIT mutations [66, 67]. The recent literature is replete with innovative strategies to identify alternative pathway inhibitors that cause cell death when combined with BCR-ABL1 TKIs. For example, our recent report on the role of STAT3 as a signaling node central to TKI resistance and the use of optimized STAT3 inhibitors with *ex vivo* activity in cells from patients with treatment-refractory CML may eventually impact other cancers lacking effective treatments [39].

The overriding primary goal in treating Ph-positive leukemia is to stay on the chronic phase side of the chronic phase/advanced disease border. Accelerated and especially blastic phase CML as well as Ph-positive ALL take on the problems and limited therapeutic options associated with more deadly diseases such as AML. The second goal is to minimize disease burden and establish durable, event-free remissions, the epitome of which is sustained, deep

molecular response. If the quantitative aspects of such a remission are aligned with attempting a carefully monitored TFR in a clinical trial setting, that once seemingly impossible horizon of 'operational cure' may be reached by a few. We view the criteria for 'operational cure', coined by the late CML pioneer John Goldman, as: a quality of life and life expectancy unaffected by CML and the absence of any intervention other than periodic monitoring of BCR-ABL1 transcript levels as specified by one's physician.

There is work to be done in Ph-positive leukemia research, most notably continuing to contend with resistance to TKIs and striving toward a cure either through eradication of CML LSCs or by better understanding and exploiting the rules for achieving operational cure with TKIs.

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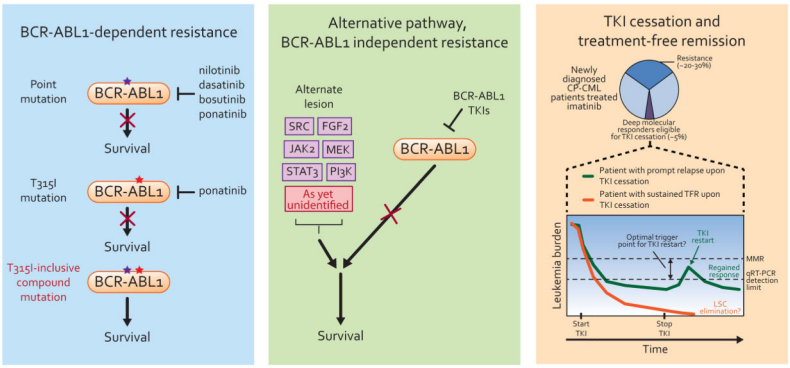
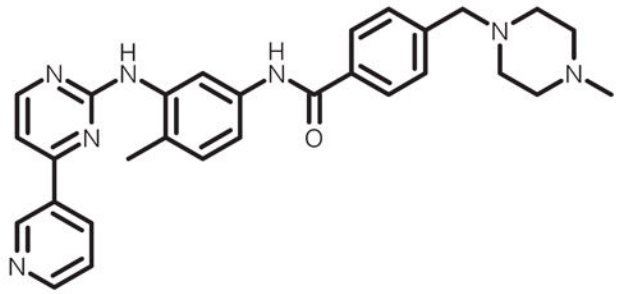
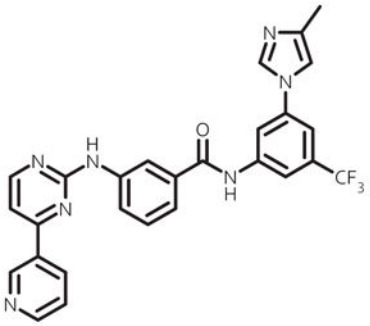
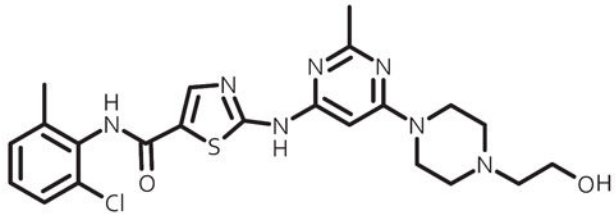


Figure 1.

Resistance to BCR-ABL1 tyrosine kinase inhibitors may take the form of (*left*) point or compound mutation-based, BCR-ABL1-dependent resistance or (*middle*) recruitment of alternative pathway signaling upon effective inhibition of BCR-ABL1. (*right*) A subset of patients achieving deep remissions on TKI therapy who elect to stop therapy subsequently demonstrate apparently durable treatment-free remission (TFR).

Table 1

FDA-approved and investigational BCR-ABL1 inhibitors targeting the kinase domain or the myristate pocket.

Inhibitor	Chemical structure	Binding site/ Inhibitor type	Regulatory status/ approval
imatinib (Gleevec)		ATP-binding site/ATP-competitive	FDA approved/Frontline therapy
nilotinib (Tasigna)		ATP-binding site/ATP-competitive	FDA approved/Frontline therapy
dasatinib (Sprycel)		ATP-binding site/ATP-competitive	FDA approved/Frontline therapy

Inhibitor	Chemical structure	Binding site/ Inhibitor type	Regulatory status/ approval
bosutinib (Bosulif)		ATP-binding site/ATP-competitive	FDA approved/2nd-line therapy
ponatinib (Iclusig)		ATP-binding site/ATP-competitive	FDA approved/2nd-line therapy
ABL001	(Currently proprietary)	Myristate pocket/Allosteric	Phase I/2nd-line therapy