Several Bruton’s tyrosine kinase inhibitors (BTKi) have been approved for the treatment of B-cell malignancies and are particularly active in chronic lymphocytic leukemia (CLL), where they have transformed the treatment paradigm. However, the activity of currently available BTKi (ibrutinib, acalabrutinib, and zanubrutinib) requires covalent bond formation with cysteine 481 (C481) of BTK; hence resistance to covalent BTKi may be mediated through mutations which remove C481. In order to overcome this resistance, and potentially prevent the proliferation of C481 mutant cells, non-covalent BTKi that do not require interaction with the C481 residue were developed. This phase Ib/II trial investigated the safety and clinical activity of vecabrutinib, a reversible, non-covalent inhibitor of BTK that inhibits both wild-type and C481-mutated BTK, in patients with advanced, BTKi-resistant B-cell malignancies. The results of the completed phase Ib dose-escalation portion of the study demonstrated that vecabrutinib was well-tolerated up to 410 mg twice daily (BID), the highest dose studied. Evidence of clinical benefit was observed, with a best response of partial response (PR) in one CLL patient and stable disease (SD) in 13 patients. Pharmacokinetics (PK) were approximately dose-proportional and sustained reductions in serum cytokine concentrations were observed at higher dose levels, suggesting BTK inhibition. However, the association between vecabrutinib dose, pharmacodynamics (PD), and clinical activity was inconsistent and the activity observed was considered insufficient for phase II expansion in patients with BTKi-resistant CLL.

Patient and disease characteristics are summarized in the Online Supplementary Table S1. Thirty-nine patients with histologically confirmed, relapsed/refractory CLL or other B-cell malignancies and ≥2 prior lines of standard systemic therapy, including progression during BTKi therapy, were enrolled and treated across seven dose-levels. The majority (77%) of patients had CLL. The enrolled population was high-risk, with a median of four prior therapies (range, 2-9), 17p deletion or TP53 mutation in 74% of all patients, and BTK C481 mutations in 55% of CLL patients.

Vecabrutinib capsules were administered orally BID at a starting dose of 20.5 mg/dose (41 mg total daily dose). Vecabrutinib was well-tolerated up to 410 mg BID (820 mg total daily dose), the highest dose studied. One patient, treated at the 41 mg BID dose level, experienced a dose-limiting toxicity (DLT), consisting of failure to receive >80% of planned vecabrutinib doses due to adverse events (AE) of grade 3 alanine aminotransferase (ALT) and grade 2 aspartate aminotransferase (AST) elevations. Upon expansion of this cohort, no additional DLT were observed and dose escalation continued. The maximum-tolerated dose of vecabrutinib was not reached.

The most common treatment-emergent AE were anemia (31%) and nausea, fatigue, headache and dyspnea (21% each). The most common AE considered treatment-related by the investigator were anemia and fatigue (10% each). Grade ≥3 AE were mainly hematologic, including anemia (23%), neutropenia (13%) and thrombocytopenia (10%) (Online Supplementary Table S2). Grade ≥3 AE considered treatment-related by the investigator consisted of leukocytosis in two patients (5.1%) and anemia, neutropenia, and increased AST in one patient (2.6%) each. No obvious pattern of dose-dependent toxicity was observed, with no grade ≥3 AE observed at the two highest dose levels. One or more serious AE (SAE) were reported in seven patients and consisted of cellulitis (in 2 patients) and lymphocytosis, intestinal perforation, myelitis, sepsis, hematuria, pleural effusion, and deep vein thrombosis (in 1 patient each). No SAE were considered related to study treatment per the investigator. Two patient deaths were associated with AE: perforated bowel in one patient treated at the 41 mg BID dose level (who had mantle cell lymphoma [MCL] with bowel involvement), and sepsis in one patient treated at the 164 mg BID dose level. Neither event was considered related to study treatment. There were no cardiac events or clinically significant electrocardiogram findings.

Vecabrutinib showed modest evidence of clinical benefit, with one PR observed in a patient with CLL (treated at the 246 mg BID dose level) and SD in 13 patients (31%; 11 CLL, 1 MCL, 1 marginal zone lymphoma). A waterfall plot of percent change in tumor burden from baseline in all patients is displayed in Figure 1. Among 14 patients with a best response of PR or SD at cycle 1, CCL3, CCL4, and TNFα eight had 17p deletion and/or TP53 mutations, and the median number of prior therapies was three (range, 2-9). Patients with PR or SD remained on study treatment for a median of 28.5 weeks (range, 6.1-56+ weeks). Eight of these patients received vecabrutinib for ≥6 months, including five who discontinued treatment due to termination of the study and not for progressive disease, suggesting some durable clinical benefit.

Pharmacokinetic data were available for 38 patients. Concentration-time profiles and linear regression analysis indicated that both exposure and median steady-state concentration, C_{ss,50}, concentrations generally increased in an approximately dose-proportional manner. Exposure to vecabrutinib was maintained across the ~12 hour dosing interval, supporting BID dosing, with cycle 1 day 8 trough values at dose levels ≥164 mg BID expected to provide >90% inhibition of BTK signaling based on an earlier single-dose phase I study.

Pharmacodynamic activity of vecabrutinib was assessed in CLL patients who completed cycle 1 (n=25) via measurement of serum cytokine levels. CCL3, CCL4, and TNFα have previously been shown to be inhibited by other BTKi. For all three cytokines, a sustained reduction in serum concentration was evident in most patients after one cycle of vecabrutinib treatment at higher dose levels (246, 328 and 410 mg), suggesting inhibition of BTK activity. Mean reductions at these dose levels ranged from 34-62% for CCL3, 33-59% for CCL4, and 24-57% for TNFα (Figure 2). In regression analyses, there was a trend for greater reduction in serum cytokine levels with increasing C_{ss,50} and AUC_{ss} as well as vecabrutinib dose, suggestive of an exposure-response effect (Online Supplementary Figure S5). Chemokine reduction was associated with clinical benefit, with decreased serum cytokine levels demonstrated in all but one patient with a clinical response of PR or SD. However, the extent of inhibition was generally less than that observed in BTKi-naïve patients treated with ibrutinib (which produced median decreases ≥80% for CCL3, CCL4 and TNFα), consistent with the limited clinical activity observed for vecabrutinib.

The question arises as to why BTK inhibition by vecabrutinib did not translate to a clinical response despite strong preclinical evidence and promising early (phase 1a) clinical PK/PD data in healthy subjects, particularly in CLL patients in whom substantial clinical activity has been observed with other non-covalent BTKi. In vitro cellular assays were performed to evaluate the half maximal inhibitory concentration (IC_{50}) values and BTK residence time (i.e., the time a BTKi remains bound to BTK) for...
vecabrutinib compared with other BTKi to look for potential correlation with outcome (Table 1). IC_{50} values for vecabrutinib (18.4 nM) and ARQ 531 (32.9 nM) were similar, however, ARQ 531 demonstrated greater clinical efficacy; conversely, fenebrutinib demonstrated greater in vitro potency (7.04 nM) but showed limited clinical activity in patients previously treated with ibrutinib.7–10 The residence time observed for vecabrutinib (15 minutes [min]) was much shorter than that observed for ARQ 531 (128 min) and fenebrutinib (557 min) and was also shorter relative to reported values for pirtobrutinib (LOXO-305; 314 min). Other possible explanations for the limited clinical activity observed are that vecabrutinib is highly protein bound (98.7%), which may have affected the availability of the free drug, or that vecabrutinib may not have been consistently distributed from blood to disease sites; either of these possibilities may have resulted in levels insufficient to provide adequate BTK inhibition. Furthermore, PK properties differ among non-covalent BTKi: the effective agents, pirtobrutinib and ARQ 531, have longer half-lives (approximately 20 hours and 55 hours, respectively) than the agents with limited clinical activity, vecabrutinib, fene-

branebrutinib and dasatinib (reported half-lives ranging between 4 and 14 hours).7,9–12 Although no single property aligned consistently with the observed clinical activity, these attributes provide possible explanations regarding the limited clinical activity observed with vecabrutinib compared to other reversible BTK inhibitors.

Overall, vecabrutinib was well-tolerated and demonstrated some evidence of clinical benefit. However, despite dose-proportional PK, the association between vecabrutinib dose, PD, and clinical response was inconsistent. Increasing the dose from 246 to 410 mg BID did not uniformly correlate with increased PD activity though there was an overall trend towards improved inhibition with dose. Assessment of clinical activity by dose may have been confounded by the impact of baseline patient characteristics: clinical benefit (i.e., PR or SD lasting >6 months) was most commonly observed in patients who were less heavily pretreated and had better prognostic factors as identified by Ahn et al.,13 such as lower baseline lactate dehydrogenase levels and wild-type TP53, regardless of dose. These results suggest that the potency of single-agent vecabrutinib was not sufficient to control disease in

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**Figure 1.** Percent change in tumor burden from baseline in patients treated with vecabrutinib. Percent change in tumor burden (sum of the product of the diameters [SPD]) from baseline at time of best response assessment is shown by patient for all patients who underwent post-treatment imaging-based disease assessment. Disease type, dose (in mg twice daily [BID]), response assessment per the Investigator, baseline molecular characteristics (Bruton’s tyrosine kinase [BTK] C481X mutation status, presence of PLCγ2 mutation, complex karyotype, TP53 mutation, or 17p deletion), and number of prior regimens received are indicated for each patient below the graph. U indicates unknown.
refractory patients; however, vecabrutinib in combination with other agents, including BCL2 inhibitors, may result in improved efficacy. Based on the dose-escalation results, the activity observed in BTKi-resistant patients at the dose levels studied was considered insufficient for phase II expansion of this patient cohort. Future directions for vecabrutinib may include indications such as chronic graft-versus-host disease or in combination with chimeric antigen receptor T-cell therapies, where dual inhibition of BTK and IL-2 Inducible T-cell Kinase (ITK) may contribute to clinical outcomes.

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Table 1. *In vitro* assessment of Bruton’s tyrosine kinase (BTK) residence time and half-maximal inhibitory concentration values for BTK engagement for vecabrutinib and other BTK inhibitors.

|           | Vecabrutinib | Ibrutinib | ARQ 531 | Fenebrutinib | Dasatinib | Pirtobrutinib |
|-----------|--------------|-----------|----------|--------------|-----------|---------------|
| BTK WT    |              |           |          |              |           |               |
| IC₅₀ for BTK engagement, nM | 18.4 | 1.65 | 32.9 | 7.04 | 34.8 | 3.7a |
| BTK residence time, minutes | 15 | >1,000 | 128 | 557 | 61 | 314a |
| BTK C481S |              |           |          |              |           |               |
| IC₅₀ for BTK engagement, nM | 34.6 | 229 | 102 | 13.1 | 78.8 | 8.5a |
| BTK residence time, minutes | 16 | 31 | 228 | 900 | 120 | 231a |

BTK: Bruton’s tyrosine kinase; BTKi: BTK inhibitor; IC₅₀: half-maximal inhibitory concentration. *IC₅₀* values and residence time values (calculated as the reciprocal of kₜ₀ [1/kₜ₀]) for pirtobrutinib (LOXO-305) are as reported by Gomez et al.; WT: wild-type.
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