ABL001 and combination

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## Disclosures of NAME SURNAME

<table>
<thead>
<tr>
<th>Company name</th>
<th>Research support</th>
<th>Employee</th>
<th>Consultant</th>
<th>Stockholder</th>
<th>Speakers bureau</th>
<th>Advisory board</th>
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ABL001 is a potent, specific inhibitor of BCR-ABL1 with a distinct allosteric mechanism of action

- Developed to gain greater BCR-ABL1 inhibition, with activity against BCR-ABL1 mutations conferring resistance to TKIs
- Potential to combine with TKIs for greater pharmacologic control of BCR-ABL1

Autoinhibition of ABL1 by engagement of myristoyl binding site

The kinase domain is normally occupied by the myristoylated N-terminus of ABL1, which serves as a key negative regulator of ABL kinase activity.
Loss of ABL1 autoinhibition due to BCR-ABL1 translocation

The fusion between BCR and ABL1 results in the loss of this regulatory element, which contributes to the constitutive activation of the kinase activity.

ABL001 allosterically inhibits BCR-ABL1 kinase activity

- ABL001 functionally mimics the role of the myristoylated peptide by occupying its vacant binding site and restoring the negative regulation of the kinase activity.

ABL001: biochemical assay at high and low ATP concentration

- ABL001 is able to inhibit ABL1 kinase regardless of high or low ATP concentration as compared to second generation TKIs.
ABL001: *In vitro* cellular activity

- Using the BaF3/BCR-ABL system that does not require IL3 to grow and is dependent on BCR-ABL for proliferation (nilotinib used as positive control):
  - ABL001 inhibited BaF3 with an IC$_{50}$ of 0.25 μM
  - If IL3 was added, the IC$_{50}$ was 2 μM (the highest dose tested)
Using the BaF3/BCR-ABL system containing point mutations, ABL001 maintained activity against all mutations, at concentrations below 50 nM.

ABL001 inhibits cells with T315I, whereas nilotinib is inactive at concentrations up to 10 μM.
ABL001 was tested in 500+ cell line panels and selectively inhibits only BCR-ABL1-positive cells with IC$_{50}$ ranging from 1–12 nM.

Cell lines that did not express BCR-ABL1 remained unaffected until the concentrations reached 2–30 μM.
KCL-22 (BC cell line) was selected to test the PK/PD relationship for ABL001.

A single oral dose of ABL001 at 3.0, 7.5, 15.0, and 30.0 mg/kg resulted in maximal pSTAT5 inhibition of 62%, 98%, 99%, and 99%, respectively.

At the 30 mg/kg dose level, >80% pSTAT5 inhibition was maintained for 16 hours post dose.

Efficacy of ABL001 in a KCL-22 xenograft model (tumor volume)

- Tumor growth inhibition:
  - 3 mg/kg corresponds to tumor growth inhibition of 55%
  - 30 mg/kg corresponds to tumor growth inhibition of 92%
FACS monitoring of the percentage of CD45+ cells per live cell in blood samples:
- A control group was treated with PBS vehicle
- 30 mg/kg corresponds to long-lasting inhibition
ABL001 and classical TKIs exhibit complementary mutation profiles

Proliferation IC$_{50}$ profiles in Ba/F3 BCR-ABL1-mutant lines

ABL001 and classical TKIs exhibit complementary mutation profiles

Myristoyl binding site mutations

Proliferation IC₅₀ profiles in Ba/F3 BCR-ABL1-mutant lines

Nilotinib

ABL001

ATP binding site mutations

Myristoyl binding site mutations

Combination of ABL001 and nilotinib prevents the emergence of resistance (KCL-22 CML xenograft)*


Dosing stopped on Day 77; all mice remain disease free >176 days
PK and metabolic profile

- In animal models (rat, dog, monkey), following oral dosing, $T_{\text{max}}$ ranged from 0.5–4 h
- Absorption is formulation-dependent
- Low to moderate bioavailability
- Binding of ABL001 to protein is high, and independent of concentration
- ABL001 is extensively distributed to most tissues
- No distribution to CNS and minimal penetration to the reproductive system
- Following administration, ABL001 is the predominant circulating form
- Biliary excretion is the major elimination pathway
- Metabolic profile different for different species (glucuronidation most readily in humans through UGT1A3, UGT1A4, UGT2B7, and UGT2B17)
- ABL001 shows reversible inhibition of CYP3A4/5, CYP2C8, CYP2C9, CYP2B6
- ABL001 is an inhibitor of BCRP, pGp, and a weak inhibitor of OCT1

BCRP, ATP binding cassette protein; CNS, central nervous system; CYP, cytochrome P450; OCT1, organic cation transporter 1; pGp, p-glycoprotein; $T_{\text{max}}$, time to maximum concentration; UGT, UDP-glucuronosyltransferase.

A single-center, open-label, randomized, crossover, two-arm study in 45 healthy subjects
- 22 subjects treated with oral formulation (variant AAA)
- 23 subjects treated with tablet formulation (variant NXA)

Both arms compared under fasting conditions, or after a low- or high-fat meal

ABL001 exhibited a negative food effect, and low- and high-fat meals decreased the bioavailability of ABL001 by 30% and 65%, respectively

ABL001 administered twice-daily was rapidly absorbed with a $T_{\text{max}}$ of 2–3 h, independent of dose

$C_{\text{max}}$ and AUC increased in an approximately dose-proportional manner

Steady state was reached before Day 15 of Cycle 1

ABL001X2101: Study design
A multicenter, Phase I, first-in-human study

**Primary Objective:** estimation of MTD/RDE

**Secondary Objectives:** safety, tolerability, pharmacodynamics, pharmacokinetic profile

Key inclusion/exclusion criteria

- **Key inclusion criteria**
  - Patients (aged ≥18 years)
  - CML in chronic, accelerated or blastic phases
  - Failed (relapsed/refractory) ≥2 prior TKIs or intolerant of TKIs
    - Patients with T315I mutation eligible after 1 prior TKI
  - ECOG performance status 0–2

- **Key exclusion criteria**
  - Strong inhibitors or inducers of CYP3A4 or CYP3A4 substrates with narrow therapeutic index
  - Laboratory parameters
    - ANC <500/mm³
    - Platelet count <50,000 mm³
    - Bilirubin >1.5 × ULN or >3.0 × ULN in patients with Gilbert’s syndrome
    - AST or ALT >3.0 × ULN
    - Creatinine >1.5 × ULN
## Demographics and baseline characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N=123</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (range), years</td>
<td>55 (23–79)</td>
</tr>
<tr>
<td>Male / female, %</td>
<td>61/ 39</td>
</tr>
<tr>
<td>ECOG PS 0–1 / 2, %</td>
<td>72/28</td>
</tr>
<tr>
<td>Prior lines of therapy, median (range)</td>
<td>3 (1–5)</td>
</tr>
<tr>
<td>1 prior TKI, %</td>
<td>5</td>
</tr>
<tr>
<td>2 prior TKIs, %</td>
<td>30</td>
</tr>
<tr>
<td>≥3 prior TKIs, %</td>
<td>65</td>
</tr>
<tr>
<td>CML-CP / -AP, / CML-BP/ALL, %</td>
<td>88/4/2/6</td>
</tr>
<tr>
<td>TKD non-mutated / mutant / not evaluable, %</td>
<td>46/30/24</td>
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</table>

### Patient disposition: single agent ABL001 in CML

<table>
<thead>
<tr>
<th>mg</th>
<th>Monotherapy BID</th>
<th>Monotherapy QD</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td>10</td>
<td>20</td>
<td>40</td>
<td>80</td>
</tr>
<tr>
<td>n</td>
<td>1</td>
<td>14</td>
<td>35</td>
</tr>
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</table>

**Median duration of exposure, weeks**

<table>
<thead>
<tr>
<th>Ongoing, n (%)</th>
<th>Discontinued, n (%)</th>
<th>Reason for discontinuation, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (100)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>14 (100)</td>
<td>5 (14)</td>
<td>2 (6)</td>
</tr>
<tr>
<td>30 (86)</td>
<td>9 (75)</td>
<td>1 (18)</td>
</tr>
<tr>
<td>29.6</td>
<td>81</td>
<td>2 (20)</td>
</tr>
<tr>
<td>52.6</td>
<td>3 (70)</td>
<td>1 (20)</td>
</tr>
<tr>
<td>69.4</td>
<td>6 (60)</td>
<td>0</td>
</tr>
<tr>
<td>16.8</td>
<td>6 (100)</td>
<td>0</td>
</tr>
<tr>
<td>51.6</td>
<td>5 (83)</td>
<td>4 (4)</td>
</tr>
<tr>
<td>53.6</td>
<td>10 (100)</td>
<td>1 (17)</td>
</tr>
<tr>
<td>37.6</td>
<td>5 (83)</td>
<td>15 (15)</td>
</tr>
</tbody>
</table>

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</table>

**Reason for discontinuation, n (%)**

<table>
<thead>
<tr>
<th>Reason for discontinuation</th>
<th>Monotherapy BID</th>
<th>Monotherapy QD</th>
<th>Total</th>
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<tbody>
<tr>
<td>AE</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pt/guardian decision</td>
<td>1 (100)</td>
<td>0</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Disease progression*</td>
<td>0</td>
<td>0</td>
<td>2 (6)</td>
</tr>
<tr>
<td>Death</td>
<td>1 (100)</td>
<td>0</td>
<td>1 (3)</td>
</tr>
</tbody>
</table>

*only 1 pt with detectable myristoil binding pocket mutations (V648H, I502L)

ABL001 pharmacokinetic profile exhibits dose proportionality from 10 to 200 mg BID.

- Rapid absorption (median $T_{\text{max}} \approx 2$ to 3 h)
- Dose-proportional increase in exposure following single and repeated dosing
- Low (<2-fold) to moderate (≈2-fold) accumulation on repeated dosing
- Short apparent elimination half-life (median≈5 to 6 h)

Dose proportionality using C1D15 (steady state) $\text{AUC}_{\text{last}}$ from individual patients: 10 to 200 mg BID.

## Safety: AE suspected of being related to study drug occurring in ≥5% of patients

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>All Grades, n (%)</th>
<th>Grade 3/4, n (%)</th>
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</thead>
<tbody>
<tr>
<td>Lipase increase</td>
<td>26 (21)</td>
<td>12 (10)</td>
</tr>
<tr>
<td>Rash</td>
<td>19 (15)</td>
<td>0</td>
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<tr>
<td>Thrombocytopenia</td>
<td>16 (13)</td>
<td>7 (6)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>15 (12)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Nausea</td>
<td>14 (11)</td>
<td>0</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>13 (11)</td>
<td>0</td>
</tr>
<tr>
<td>Amylase increased</td>
<td>12 (10)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Headache</td>
<td>12 (10)</td>
<td>0</td>
</tr>
<tr>
<td>Pruritus</td>
<td>11 (9)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Anemia</td>
<td>9 (7)</td>
<td>5 (4)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>9 (7)</td>
<td>0</td>
</tr>
<tr>
<td>Myalgia</td>
<td>9 (7)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>9 (7)</td>
<td>0</td>
</tr>
<tr>
<td>Hypophosphatemia</td>
<td>7 (6)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>7 (6)</td>
<td>5 (4)</td>
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Safety: dose-limiting toxicities

• 92 patients evaluable for dose escalation

• There were 6 dose-limiting toxicities:
  
  – Grade 3 lipase increase (n = 3; 40 mg BID, 200 mg QD, ABL001 40 mg BID + dasatinib 100 mg QD)
  
  – Grade 2 myalgia/arthralgia (80 mg BID)
  
  – Grade 3 acute coronary event (150 mg BID)
  
  – Grade 3 bronchospasm (200 mg BID)

• MTD not declared; 40 mg BID declared as recommended dose for single-agent BID schedule in CML-CP
  
  – Based on combined analyses of safety, preliminary efficacy, and results of a population-based PK-response model

Responses with single agent asciminib BID ≥ 3 mos exposure on study

In vitro and in vivo activity against T315I mutation

• Sensitivity of parental KCL-22WT, KCL-22 T315I and KCL-22 A337V to ABL001 and Nilotinib

• KCL-22 T315I were implanted in a xenograft model and ABL001 tested at increased dose

Responses in CML patients with T315I mutation

- 11 of 77 (14%) CML patients treated with BID ABL001 had T315I mutations
- ABL001 exhibits a similar duration of exposure in CML patients regardless of T315I mutation status
- Responses in T315I mutant CML patients treated with single agent ABL001 BID for ≥ 3 months
  - 4 of 10 patients in cytogenetic relapse at baseline (> 35% Ph+) achieved CCyR by 6 mo
  - 6 patients have maintained stable disease without achieving CCyR or MMR
  - 1 patient has maintained a baseline MMR for > 1 year
  - No patients have progressed to blast crisis

Possible mechanisms of resistance to asciminib (I)

- **Upregulation of the ABCG2 efflux pump**
  - Generation of 5 asciminib resistant cell lines
  - Asciminib level was measured by mass spectrometry
  - Asciminib was undetectable in K562 asciminib-R
  - ABCG2 inhibitor (Ko143) restored asciminib effectiveness against K562 asciminib-R
  - Asciminib resistance can be override by dose escalation of the drug or the association with ABCG2 inhibitor
Possible mechanisms of resistance to asciminib (II)

- Emergence of BCR-ABL1 mutations at the myristoyl binging site and at a distant residue
  
  - C464W as asciminib-resistant mutant: the bulky tryptophan residue prevents access of asciminib to the myristoyl-binding pocket
  
  - Other mutations near the myristoyl-binding pocket that can confer resistance are: A337V, P465S, V468F or compound mutation M244V/A337V
ABL001 overcomes TKI resistance and enhances MDM2 inhibitor activity in blast crisis

- ABL001 exhibits cytotoxicity in cell from BC patient samples with multiple mutations treated with various TKIs

- Activation of p53 by MDM2 inhibition induces apoptosis and enhances the activity of ABL001 in apoptosis induction in CD45+, CD34+CD38+ or CD34+CD38- cells

- ABL001 overcomes BCR-ABL TKI resistance and enhances MDM2 inhibitor activity in BC-CML
Clinical case (1): our first and long-term treatment pts

- Previously resistant to imatinib, nilotinib and dasatinib. Also intolerant to dasatinib 100 mg, with several episodes of hematologic toxicity (Grade 3 thrombocytopenia)
Clinical case (2): a patient who developed resistance to ABL001 but was rescued with dose escalation

- Previously resistant to imatinib (ACA/OCA) and dasatinib (F317V, also intolerant to dasatinib with neutropenia, mouth ulcers). Previous thrombotic events.

- Start ABL001 40 mg BID

- Increase to 80 mg BID

- A337T mutation

- MMR (3 logs bcf)

- MR4.5

- BCR-ABL / ABL % (IS)

- Cumulative results show the most recent 6 visits with valid results. Please consult previous reports for results from earlier visits not shown.
ABL001 vs bosutinib in CML pts previously treated with 2 or more TKIs

• Primary endpoint: to compare the rate of MR3 at 24 weeks
Phase II Study Design – Asciminib add on to 1L Imatinib (CABL001E2201)

4 Arm Study Design Allows Evaluation of 2 dose levels against 2 controls

- CML-CP patients
- ≥2 years on frontline Imatinib
- BCR-ABL >0.01%--≤1.0% (suboptimal response)

• Asciminib 40 mg + Imatinib
• Asciminib 60 mg + Imatinib
• Stay on Imatinib
• Switch to Nilotinib

Primary Analysis

MR 4.5 at wk 48

Primary Objective
• Compare MR4.5 rate at 48 weeks with asciminib (40 or 60 mg) + imatinib vs continued imatinib

Secondary Objective
• Estimate difference in MR4.5 rate at 48 weeks between asciminib (40 or 60 mg) + imatinib and switch to Nilotinib
• Assess additional efficacy parameters with asciminib (40 or 60 mg) vs continued imatinib switch to nilotinib
• Safety and tolerability profile of Asciminib + Imatinib vs continued Imatinib or switch to Nilotinib
• Assess PK profile of Asciminib (40 or 60 mg) + Imatinib

Exoloratory Objective
• Patient–reported outcomes
• Biomarkers

* Patients on imatinib continuation without MR4.5 after 48 weeks of treatment will be offered to crossover to combination treatment
Conclusions

- ABL001 was generally well tolerated in heavily-treated CML patients resistant to or intolerant of prior TKIs.
- Preliminary pharmacokinetic exposures appear linear in the dose range tested.
- Evidence of single-agent efficacy at 40 mg BID.
  - Clinical activity across several TKI-resistant mutations (e.g., V299L, F317L, Y253H).
  - Myristoyl binding pocket mutations (V468H, I502L, A337V, C464W) may lead to clinical resistance.
- Allosteric inhibition of BCR-ABL1 is a promising therapeutic approach in patients with CML.