**Background**

Polo-like Kinase 1 (PLK1):
- Serine/threonine kinase, master regulator of cell cycle progression
- Inhibition of PLK1 causes mitotic arrest in prometaphase and subsequent cell death
- PLK1 is overexpressed in numerous cancer types, including AML, and associated with poor prognosis

**Onvansertib**:
- A pan-PLK inhibitor, cohorts in combination with LDAC, improved survival in a randomized Phase 2 trial in AML patients

**3 Generation PLK inhibitor**, with increased specificity, potency and pharmacokinetic properties was needed to optimize features that hampered future development of valiturnt

**Onvansertib (also known as FCM-073 and NMS-1288507):**
- Oral/cytoplasmic, highly selective PLK inhibitor
- 24-hour half-life
- Induces G2/M arrest and apoptosis in cancer cells, including leukemic cells
- Inhibits tumor growth alone and in combination with Cytarabine in AML mouse models
- Safe and well tolerated (Phase 1 dose escalation trial in patients with solid tumors)

Current open-label trials with Onvansertib (AML) - NCT02860130 and metastatic Castration-Resistant Prostate Cancer (mCRPC) - NCT03161842

**Phase 1b/2 Trial Design and Objectives**

- **Study design:**
  - **Dosing schedule:**
    - Onvansertib 1x 5 days + either low LDAC or in Combination with LDAC (20 mg/m² for a 5g over a 21 to 28- day cycle)
  - **Phase 1b: Dose escalation in 1x 5 days + expansion cohort at MTD or RD2 for Phase 2**
  - **RD2:** Incremental dose increase in subsequent cohorts of 3 patients
  - **Dose limiting toxicity (DLT) evaluated during the 1st cycle**

- **Study Objectives:**
  - **Primary objectives:**
    1. Safety: and define dose-limiting toxicity (DLT) and maximum tolerated dose (MTD) in combination with Cytarabine (MTD or RD2)
  - **Secondary objectives (LDAC):**
    1. Assess incidence and severity of adverse events
    2. Analyze pharmacokinetics
    3. Exploratory objectives (LDAC):
      1. Evaluate pharmacodynamic and diagnostic biomarkers associated with response to treatment

**Key Eligibility Criteria:**
- Patients with AML who have received ≥3 Phase 1/2 or Phase 2 previous therapies or treatment/relapse-patients ineligible for induction therapies (Phase 1b and 2)
- Treatment-related AML or M4 are excluded

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**Pharmacokinetics**

- The exposure (AUC) following Onvansertib administration was comparable in combination with either LDAC or Decitabine
- AUC on day 8 showed an accumulation of almost 2-fold between day 1 and 5

**Safety**

- **Primary endpoint of Phase 1b**
  - No trial therapy related deaths
  - No SAEs were considered related to study drug treatment

**Hematological Adverse Events Reported in ≥ 10% Patients (N=19)**

<table>
<thead>
<tr>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
</tr>
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<tbody>
<tr>
<td>Neutropenia</td>
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<td>2/8</td>
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<tr>
<td>Anemia</td>
<td>14/9</td>
<td>4/10</td>
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<tr>
<td>Thrombocytopenia</td>
<td>0/0</td>
<td>3/6</td>
<td>1/1</td>
</tr>
<tr>
<td>Dizziness</td>
<td>1/1</td>
<td>2/4</td>
<td>0/0</td>
</tr>
<tr>
<td>Constipation</td>
<td>0/0</td>
<td>2/4</td>
<td>0/0</td>
</tr>
</tbody>
</table>

- AEs possibly related to Onvansertib
  - Grade 2 nausea in 4 patients
  - Grade 1 nausea in 4 patients
  - Grade 1 vomiting in 1 patient
  - Grade 1 diarrhea in 1 patient
  - Grade 1 constipation in 1 patient

- No SAEs were considered related to study drug treatment

**Patient Demographics**

<table>
<thead>
<tr>
<th>Patient</th>
<th>AML subtype</th>
<th>FAB</th>
<th>Age</th>
<th>Gender</th>
<th>Race</th>
<th>Prior treatment</th>
<th>Prior LDAC</th>
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<tr>
<td>P005</td>
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<td>M4</td>
<td>65</td>
<td>Male</td>
<td>White</td>
<td>0</td>
<td>0</td>
<td>24</td>
</tr>
</tbody>
</table>

**Anti-Leukemic Activity**

- **Phase 1 objective is to assess the safety and tolerability of Onvansertib in combination with standard-of-care chemotherapy**
- **Phase 2 objective is to assess the safety and tolerability of Onvansertib in combination with standard-of-care chemotherapy**
- Of the 10 patients evaluable for safety, 12 patients had an evaluable bone marrow biopsy to assess anti-leukemic activity based on criteria from the 2013 ELN recommendations
- Of the 12 patients evaluated for preliminary anti-leukemic activity, 1 patient had a PR, 9 patients had stable disease and 2 patients had PD

**Biomarker Strategy and Analyses**

- PLX1 inhibition can be monitored in chronic myeloid leukemia (CML)
- pCTP as a marker of PLX1 activity
  - PKS phosphorylates the translational control tumor protein (ECTP) on serine 46
  - pCTP was identified as a specific marker for PLX1 activity in vivo in preclinical models

Assessment of PLX1 activity in patients through pCTP:
- Blood samples were collected from patients enrolled in the trial in day 1 of treatment (pre-dose and 1h post-dose) and on day 4 (dose of Onvansertib)
- pCTP and TCTP were assayed using Western-Blot and LC/MS/MS (pCTP/TCTP) was quantified
- A target of ≥10% decrease in pCTP at 1h post-dose compared to pre-dose (ำ 50%) and this decrease was sustained on day 4 of treatment

**Conclusion and Perspectives**

- PLX1 inhibition was not dependent on Onvansertib's dose or PK, either in the combination treatment

**Conclusions and Perspectives**

- JMPR TERT inhibition was observed in 5 out of 15 patients and was associated with a higher response to treatment, measured by decreases in circulating and bone marrow blasts
- Implementation of a pCTP biomarker strategy going forward will increase the opportunity to identify patients most likely to respond to Onvansertib

**Phase 1b/2 Trial of Onvansertib in Combination with either Low-Dose Cytarabine or Decitabine in Patients with t(8;21) Acute Myeloid Leukemia**

- **Eligible Patient**
  - t(8;21)-AML patient:
    - Bone marrow samples obtained at baseline and at end of cycles 1, 2, 4, 5 and 12 % blasts assessed by IHC by clinical site
  - **Target Engagement**
    - Onvansertib 12mg/m²
    - Decitabine 5mg/m²
    - LDAC (40 mg/m² for a 5g over a 21 to 28- day cycle)

- **PLX1 inhibition by Onvansertib (Target engagement) is correlated with higher response to treatment**
- **Methods:**
  - Blood samples collected on day 1 (pre-dose), 5, 12, 21 of cycle and 4 of circulating blasts assessed by flow cytometry
  - Bone marrow samples obtained at baseline and at end of cycles 1, 2, 4 and 12 % blasts assessed by IHC by clinical site
  - **Results:**
    - Of the 15 patients with target engagement showed a decrease in circulating blasts of ≥20% on the last time point recorded compared to baseline
    - 4 out of 5 patients with target engagement had a decrease of ≥50 % in their t(8;21) MM blasts compared to baseline
    - Decreases in circulating and bone marrow blasts were significantly higher in patients with target engagement compared to patients without target engagement

**Target Engagement**

- **No Target Engagement**

- **Target Engagement**

- **Biomarker Strategy and Analyses**
  - **PLX1 inhibition can be monitored in patients through pCTP status**
  - **pCTP as a marker of PLX1 activity**
    - PKS phosphorylates the translational control tumor protein (ECTP) on serine 46
    - pCTP was identified as a specific marker for PLX1 activity in vivo in preclinical models
  - **Assessment of PLX1 activity in patients through pCTP:**
    - Blood samples were collected from patients enrolled in the trial in day 1 of treatment (pre-dose and 1h post-dose) and on day 4 (dose of Onvansertib)
    - pCTP and TCTP were assayed using Western Blot and LC/MS/MS (pCTP/TCTP) was quantified
    - A target of ≥10% decrease in pCTP at 1h post-dose compared to pre-dose (้ำ 50%) and this decrease was sustained on day 4 of treatment
    - PLX1 inhibition was not dependent on Onvansertib's dose or PK, either in the combination treatment
  - **Conclusion and Perspectives:**
    - PLX1 inhibition by Onvansertib (Target engagement) is correlated with higher response to treatment