PRT2527 is a potent and selective CDK9 inhibitor that demonstrates anti-cancer activity in preclinical models of hematological malignancies and solid tumors with MYC amplification

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Introduction

- CDK9 phosphorylates RNA Pol II and regulates transcription
- Regulates expression of several immediate early genes driving oncogenesis and resistance (i.e. MYC, MYB, MCL1)
- Non-selective CDK9 inhibitors have demonstrated clinical activity in multiple tumor types but poor tolerability
  - Lack of selectivity and potency vs other CDK9 inhibitors is believed to contribute to low therapeutic window
PRT2527 is a Potent and Selective CDK9 Inhibitor

- PRT2527 is a potent CDK9 inhibitor with a biochemical IC\textsubscript{50} value of 0.98 nM at 1 mM ATP and a protein binding adjusted potency of 198 nM determined by a human plasma In Cell Western (ICW) assay.

- PRT2527 is highly selective among CDK family members and across 177 kinases. Biochemical IC\textsubscript{50} values for each CDK members were determined at 1 mM ATP and fold selectivity over CDK9 was derived. Kinome screening was conducted with 200 nM PRT2527 at 1 mM ATP.

### Table: PRT2527 and Other Compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>AZD4573</th>
<th>KB0742</th>
<th>VIP152**</th>
<th>PRT2527</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDK9</td>
<td>1.9</td>
<td>483</td>
<td>16</td>
<td>0.98</td>
</tr>
<tr>
<td>Proliferation* IC\textsubscript{50} (nM)</td>
<td>11</td>
<td>915</td>
<td>84</td>
<td>18</td>
</tr>
<tr>
<td>Plasma* IC\textsubscript{50} (nM)</td>
<td>162</td>
<td>986</td>
<td>923</td>
<td>198</td>
</tr>
</tbody>
</table>

| CDK1     | 23x     | >20x   | 371x     | 73x     |
| CDK2     | 35x     | >20x   | 147x     | 340x    |
| CDK3     | 2x      | >20x   | 37x      | 35x     |
| CDK4     | 53x     | >20x   | 38x      | 250x    |
| CDK5     | 37x     | >20x   | >600x    | >1000x  |
| CDK6     | 79x     | >20x   | 296x     | >1000x  |
| CDK7     | 150x    | >20x   | >600x    | >1000x  |

*Internal data: biochemical assay at 1 mM ATP, H929 CTG proliferation assay
**VIP151 was formerly BAY151 and licensed to Vincera by Bayer
PRT2527 Treatment Depletes MCL1, MYC, and Induces Apoptosis *In Vitro* and *In Vivo*

- PRT2527 depletes pSer2RNAP2 and onco-proteins with short half-lives such as MCL1, MYC, and induces apoptosis in a dose-dependent manner in MV-4-11 cells after 4 hours treatment (A). MCL1 was identified as one of the major down-regulated proteins in an unbiased differential proteomic analysis (B. MV-4-11 cells, 4 hours treatment).

- IV administration of PRT2527 at 15 mg/kg q2h in mice achieved coverage above human plasma IC_{50} for 6 hours in tumor (C, red line), temporally depleted pSer2RNAP2, MCL1, MYC, and induced apoptosis as measured by cleaved caspase 3 activation (D) in tumor tissue. Study was conducted in a MV-4-11 subcutaneous xenograft model.
PRT2527 Induces Cell Death in Hematological and Solid Tumor Models *In Vitro* and *Ex Vivo*

PRT2527 anti-proliferative potencies in hematological (A) and solid tumor cell lines (C) were determined via 1-day and 2-day assays, respectively. For primary cultures of heme (B) and solid tumors with *MYC* amplification (D), anti-proliferative IC$_{50}$ values were determined through a 2-day proliferation assay.

ACC: adenoid cystic carcinoma  
ALL: acute lymphoblastic leukemia  
AML: acute myeloid leukemia  
CML: chronic myelogenous leukemia  
CRPC: castrate-resistant prostate cancer  
DLBCL: diffuse large B-cell lymphoma  
HL: Hodgkin’s lymphoma  
NSCLC: non-small cell lung cancer  
PDAC: pancreatic ductal adenocarcinoma
PRT2527 is Efficacious in CDX and PDX Models of Heme and Solid Tumors with MYC Alterations

- Intermittent IV administration of PRT2527 (2on/5off or QW) inhibited tumor growth in AML MV-4-11 model (A) and DH-DLBCL Karpas-422 model carrying MYC translocation (B). PRT2527 was also efficacious in various PDX models with MYC amplification (C, D). PRT2527 efficacy in additional CDX and PDX models bearing MYC translocation or amplification are summarized in Table E.

**Table E**

<table>
<thead>
<tr>
<th>Model #</th>
<th>Tumor type</th>
<th>MYC alter.</th>
<th>TGI (%)</th>
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<tbody>
<tr>
<td>SU-DHL-4</td>
<td>TH-DLBCL/CDX</td>
<td>Translocation</td>
<td>126</td>
</tr>
<tr>
<td>PNX-0223</td>
<td>DH-DLBCL/PDX</td>
<td>Translocation</td>
<td>84</td>
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<tr>
<td>PNX-0019</td>
<td>Esophageal/PDX</td>
<td>Amp. (36x)</td>
<td>49</td>
</tr>
<tr>
<td>CTG-2271</td>
<td>NSCLC/PDX</td>
<td>Amp. (23x)</td>
<td>63</td>
</tr>
<tr>
<td>ACCX9</td>
<td>ACC/PDX</td>
<td>Overexpression</td>
<td>50</td>
</tr>
</tbody>
</table>

**Figure A**

- AML: MV-4-11 CDX

**Figure B**

- DH DLBCL: Karpas-422 CDX

**Figure C**

- PNX-0001 PDAC PDX

**Figure D**

- CTG-2308 Breast PDX
PRT2527 reduces MYC transcriptional activity in a CRPC MYC-reporter model *in vitro* and *in vivo*. MYC-DU145 cells carry a firefly luciferase reporter gene controlled by a MYC responsive element. MYC transcription activity fully recovered 24h after IV administration (C).

- PRT2527 inhibited MYC transcriptional activity in a CRPC MYC-DU145 model *in vitro* (A, 24h) and *in vivo* (B, C). MYC-DU145 cells carry a firefly luciferase reporter gene controlled by a MYC responsive element. MYC transcription activity fully recovered 24h after IV administration (C).

- Intermittent IV administration of PRT2527 (15 mg/kg q2h, QW) significantly inhibited tumor growth of a CRPC PDX model (LuCap 35) *in vivo* (D). **: $P < 0.01$; ***: $P < 0.001$; ****: $P < 0.0001$
Significant Survival Benefit of PRT2527 in Responsive Pancreatic and Gastroesophageal PDX Models with MYC Amplification

- Intermittent IV administration of PRT2527 (15 or 30 mg/kg QW) significantly extended median survival of mice bearing PDX models of PDAC (A) and gastroesophageal (B) with MYC amplification.
PRT2527 is a potent and highly selective CDK9 inhibitor

PRT2527 treatment depletes oncogenic drivers with short half-lives such as MYC and MCL1 and induces apoptosis

Intermittent IV administration of PRT2527 demonstrated strong efficacy in hematological malignancies and solid tumors models with MYC amplification and prostate cancer with AR dependence

PRT2527 IND enabling studies completed and PRT2527 is expected to enter the clinic in Q4 for heme and solid tumors with MYC amplification