Development and evaluation of a cardiac glycoside extract, PBI-05204, as a novel anticancer agent

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Background: Rationale

Na, K-ATPase

- Transmembrane protein
- Four isoforms of $\alpha$ subunits (binding site for cardiac glycoside)
- Three isoforms of $\beta$ subunits
- One $\gamma$ subunit
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**Concentration (nM)**

0 100 200 300 400 500 600

Percent growth of control cells

0 20 40 60 80 100 120

**IC$_{50}$**

- Panc-02
- BXPC3
- MiaPaca
- PANC-1

**α 1**

**α 3**

**β-Actin**

**Mouse**
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- $\alpha_1$
- $\alpha_3$
- $\beta$-Actin

**Mouse**

- Panc-02
- BxPC3
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**Human**

- Panc-02
- BxPC3
- MiaPaca
- PANC-1
Oleandrin-mediated inhibition of human tumor cell proliferation: Importance of Na,K-ATPase α subunits as drug targets

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Abstract
Cardiac glycosides such as oleandrin are known to inhibit the Na,K-ATPase pump, resulting in a consequent increase in calcium influx in heart muscle. Here, we investigated the effect of oleandrin on the growth of human and mouse cancer cells in relation to Na,K-ATPase subunits. Oleandrin treatment resulted in selective inhibition of human cancer cell growth but not rodent cell proliferation, which corresponded to the relative level of Na,K-ATPase α3 subunit protein expression. Human pancreatic cancer cell lines were found to differentially express varying levels of α3 protein, but rodent cancer cells lacked discernable expression of this Na,K-ATPase isoform. A correlation was observed between the ratio of α3 to α1 isoforms and the level of oleandrin uptake during inhibition of cell growth and initiation of cell death; the higher the α3 expression relative to α1 expression, the more sensitive the cell was to treatment with oleandrin. Inhibition of proliferation of Panc-1 cells by oleandrin was significantly reduced when the relative expression of α3 was decreased by knocking down the expression of α3 isoform with α3 siRNA or increasing expression of the α1 isoform through transient transfection of α1 cDNA to the cells. Our data suggest that the relative lack of α3 (relative to α1) in rodent and some human tumor cells may explain their unresponsiveness to cardiac glycosides. In conclusion, the relatively higher expression of α3 with the limited expression of α1 may help predict which human tumors are likely to be responsive to treatment with potent lipid-soluble cardiac glycosides such as oleandrin.
PBI-05204, is a supercritical CO$_2$ extract of organically grown *Nerium oleander* containing oleandrin, a cardiac glycoside, which inhibits Na-K ATPase pump activity through the Na,K-ATPase α-3 subunit.

Over-expression of the α-3 subunit in malignant cells strongly correlates with tumor proliferation.

Our published research has documented that oleandrin:
• PBI-05204, is a supercritical CO₂ extract of organically grown *Nerium oleander* containing oleandrin, a cardiac glycoside, which inhibits Na-K ATPase pump activity through the α-3 subunit.

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✓ **Inhibits FGF-2 export** through membrane interaction with the Na-K ATPase pump.
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- **Inhibits FGF-2 export** through membrane interaction with the Na-K ATPase pump.
- **Inhibits activation of NF-κB** and causes cell death by inducing Fas expression in tumor cells; forms autophagosomes.
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- Inhibits phosphorylation of Akt, concomitantly increasing MAPK expression; both indicating impending cell injury and death.
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- **Inhibits phosphorylation of Akt**, increased MAPK expression; both indicating impending cell injury and death.

- **Down regulates mTOR effector protein phosphorylation**, and expression of p70S6K and S6.
PBI-05204: Oleandrin and lipid soluble cardiac glycosides

Na, K-ATPase

MAPK

ERk1/2

AP-1

PI3k

ROS

Glycolysis

NF-Kb

bFGF

HIF1α

Akt

P21cp1

Cell cycle arrest

Autophagic cell death

Apoptosis

Red cross – Down-regulation; Blue arrow – Up-regulation; Dashed arrow – hypothesized down regulation
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MAPK
ERk1/2
AP-1
P21cp1
Cell cycle arrest

bFGF

Control
Oleandrin 10 nM

G1
17.1% G2/M

G1
35.1% G2/M

DNA Content
Cell Number

Phoenix BIOTECHNOLOGY, INC.
PBI-05204: Oleandrin and lipid soluble cardiac glycosides

Na, K-ATPase

- MAPK
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- AP-1
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Integrative Cancer Therapies 6(4); 2007
PBI-05204: Oleandrin and lipid soluble cardiac glycosides

Na, K-ATPase

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- AP-1
- P21<sup>cp1</sup>

PI3k
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ROS

Glycolysis

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Cell cycle arrest

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An Open Label Phase I Trial of PBI-05204 in Advanced Cancer Patients

- **Primary Objectives**
  - To determine the maximum tolerated dose (MTD) or biologically effective dose (BED) based on pharmacodynamic markers.
  - To evaluate pharmacokinetic characteristics.

- **Secondary Objectives**
  - To assess initial response rate.
  - To examine target Na, K- ATPase subunit expression in tumor tissues
  - To evaluate drug-mediated modulation of pharmacodynamic markers in PBMCs (pAkt, mTOR inhibition)
• Conventional 3+3 Phase I dose escalation.
• PBI-05204 administered orally for 21 of 28 day cycle.
• Starting dose = 0.0083 mg NOE/kg (0.2 µg/kg oleandrin)
• Correlative studies including PKs, optional biopsies and PBMCs.
• EKG’s, 24-hour Holter monitor and Echo performed.
• Dose increased by 100%, by 50% if a grade 2 AE occurred. If no other grade 2 AE, resumed at 100%.
• Patients on study until tumor progression or unacceptable toxicity.

• Key eligibility criteria:
  • ≥18 yrs old, metastatic or locally advanced primary solid malignancies who are not candidates for standard therapy.
  • Measurable disease, as defined by RECIST.
  • ECOG PS ≤ 1. Adequate hematologic, renal, & hepatic function.
  • Symptoms of brain mets ruled out by CT/MRI and/or fully treated.
Cycles are 4 weeks each

<table>
<thead>
<tr>
<th>Days -7 to -1</th>
<th>Week 1 Days 1-7</th>
<th>Week 2 Days 8-14</th>
<th>Week 3 Days 15-21</th>
<th>Week 4 Days 22-28</th>
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<tbody>
<tr>
<td>Biopsy PKs</td>
<td>PBI-05204 po</td>
<td>PBI-05204 po</td>
<td>PBI-05204 po</td>
<td>Biopsy</td>
</tr>
<tr>
<td>ECG, 24 hr</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Holter, ECHO</td>
<td></td>
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</tr>
</tbody>
</table>

Day 1
- PKs, PDs, ECG

Day 2
- PKs, ECG

Day 7
- PDs, 24 hr Holter
- Day 8 PKs, ECG

Day 15
- PKs, ECG

Day 21
- PKs, PDs

1 PBI-05204 given daily (through cohort 5) or twice daily as a divided dose (cohort 6+)
2 for patients with cardiomyopathy who have not had an echocardiogram three months prior
3 Taken pre-dose
4 Scheduled for Day 7 ± 2 days
5 Scheduled for Day 21 ± 2 days
6 Scheduled for any time during Days 15-21
7 Only for subjects on QD dosing
Summary of Cardiac Adverse Events

• No cardiac adverse event ≥ grade 2 observed.
• Only grade 1 adverse event seen in 5/22 patients (23%).
• EKG and 24-hour Holter monitor changes observed were minor, inconsistent and not clinically significant.
• Observed drug related adverse events –
  1. First degree Atrioventricular block, palpitations, hypertension in 2/22 patients (9% each)
  2. Supraventricular tachycardia, ventricular extrasystole in 1/22 patients (5% each)
  3. EKG abnormality- QTc prolongation in 1/22 patients (5%).
Progressive disease
Stable disease

20/22 patients evaluable by RECIST
* - 233% increase
# 2/22 - progression with new metastases
1/22 - clinical progression
1/22 - non-measurable disease
Summary of Responses

- Stable disease was seen in 9/20 patients (45%) with various tumor types after first restaging (2 months).

- Out of these, minor response was seen in 3 patients—one each with colorectal (17% decrease), bladder (11% decrease) and fallopian tube cancer (10% decrease).

- The longest duration of stable disease was 6 months in a colorectal cancer patient.
Pharmacokinetic Summary

• Samples collected on Day 1, 8, 15, and 21 of Cycle 1.

• A dose-dependent increase was observed in mean peak plasma oleandrin concentration. (0.93 to 2.41 ng/ml).

• No cardiac-related toxicity within this plasma concentration range.

• A trend was observed for a dose-dependent increase in Mean AUC.

• Compared to digoxin ($T_{1/2\gamma} = 36$hrs), mean oleandrin $T_{1/2\beta}$ was relatively short (6 to 14 hours).
Oleandrin Dose vs. AUC

Mean Oleandrin Dose (µg/kg/day)

Oleandrin AUC (µg/L*hr)

0.3 QD
0.63 QD
1.4 QD
2.1 QD
3.0 (div BID)
4.7 (div BID)
<table>
<thead>
<tr>
<th>Cohort Sched</th>
<th>Mean PBI-05204 Dose (mg NOE/day)</th>
<th>Mean Oleandrin Dose (ug/kg/day)</th>
<th>Mean Oleandrin Cmax* (Cycle 1 Day 8) (ng/ml)</th>
<th>ke (hr⁻¹)</th>
<th>V (L/kg)</th>
<th>Cl (L/hr/kg)</th>
<th>t_{1/2B} (hr)</th>
<th>AUC (ug/L*hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1.2</td>
<td>0.30</td>
<td>0.51</td>
<td>0.098</td>
<td>54.3</td>
<td>5.31</td>
<td>7.08</td>
<td>4.7</td>
</tr>
<tr>
<td>3</td>
<td>2.2</td>
<td>0.63</td>
<td>0.93 (0.76 - 1.03)</td>
<td>0.163 ± 0.12 (74.3)</td>
<td>0.58 ± 0.29 (50.7)</td>
<td>0.071 ± 0.02 (25.0)</td>
<td>6.44 ± 4.9 (75.3)</td>
<td>9.5 ± 3.7 (38.6)</td>
</tr>
<tr>
<td>4</td>
<td>5.1</td>
<td>1.39</td>
<td>2.19 (1.44 - 2.77)</td>
<td>0.290 ± 0.26 (89.6)</td>
<td>0.62 ± 0.23 (36.5)</td>
<td>0.14 ± 0.11 (74.5)</td>
<td>5.84 ± 5.2 (88.8)</td>
<td>16.4 ± 12.2 (74.4)</td>
</tr>
<tr>
<td>5</td>
<td>7.8</td>
<td>2.09</td>
<td>1.93 (0.71 - 3.27)</td>
<td>0.071 ± 0.03 (35.8)</td>
<td>1.79 ± 1.26 (70.4)</td>
<td>0.11 ± 0.06 (50.8)</td>
<td>10.7 ± 4.3 (40.1)</td>
<td>24.2 ± 17.3 (71.5)</td>
</tr>
<tr>
<td>6</td>
<td>10.0</td>
<td>3.04</td>
<td>1.26 (0.81 - 1.71)</td>
<td>0.065 ± 0.04 (60.1)</td>
<td>4.12 ± 3.26 (79.1)</td>
<td>0.26 ± 0.18 (70.7)</td>
<td>13.3 ± 7.2 (54.2)</td>
<td>20.8 ± 20.2 (97.1)</td>
</tr>
<tr>
<td>7</td>
<td>22.0</td>
<td>4.71</td>
<td>2.38 (1.27 - 3.19)</td>
<td>0.052 ± 0.01 (25.4)</td>
<td>3.04 ± 1.47 (48.5)</td>
<td>0.16 ± 0.11 (65.0)</td>
<td>14.0 ± 4.1 (47.4)</td>
<td>36.3 ± 17.5 (48.2)</td>
</tr>
</tbody>
</table>

*measured at 2 hrs
Western blot analysis of PBMC in one patient from cohort 3, 5 and 6 patients. Down regulation of pAkt and mTOR effector, pp70S6K and pS6 were observed in all of these three patients.
Summary of Pharmacodynamic analysis

- Western blot analysis in PBMCs showed a trend toward reduction of phosphorylation of Akt, p70S6K, and S6 in a time and dose dependent manner suggesting PBI-05204 is capable of inhibiting oncogenic cell signaling via PI3-kinase/mTOR pathways.

- Continuing assessment of Na, K-ATPase α-3 subunit expression with respect to reduction of PI3 kinase/mTOR protein expression is ongoing.
Conclusions

• PBI-05204 is well tolerated up to 0.2255 mg/kg dose.
• No grade 3 or higher adverse events noted.
• PD analysis has shown a trend toward reduction of phosphorylation of Akt, p70S6K, and S6.
• PK analysis has shown a dose dependent increase in mean plasma oleandrin concentration.
• No significant cardiac adverse events have been observed.
• Activity has been shown in diverse tumor types.
• A lower dose is now being explored as a possible recommended BED for patients of selected tumor types.
Acknowledgements

MD Anderson Cancer Center

- Dr David Hong
- Dr Razelle Kurzrock
- Dr Gerald Falchook
- Dr Aung Naing
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**Phoenix Biotechnology**
- Crandell Addington

• All our patients, their families, and caregivers
Adverse Events

• No grade 3 AEs observed through Cohort 7.

• Common drug-related AEs: abdominal pain (27%), diarrhea, myalgias, fatigue (18% each), nausea, constipation (14% each).

• No cardiac adverse event ≥ grade 2 observed. No clinically significant EKG or 24-hour Holter monitor changes.

• Grade 3 proteinuria and fatigue observed at Cohort 8. Currently enrolling a second 3 patient cohort at the MTD (Cohort 7).

Best Response

• Stable disease in 9/20 patients (45%) at first restaging (2 months). Of these, 3 with minor response: colorectal (17% red), bladder (11% red), fallopian tube (10% red).

• The longest duration of stable disease was 6 months (colorectal).