Disclosures

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Employee of Onyx Pharmaceuticals
Potent Inhibition of Multiple Proteasome Subunits by Carfilzomib in Solid Tumor and Multiple Myeloma Patients


Onyx Pharmaceuticals, South San Francisco, CA
The Ubiquitin Proteasome Pathway

Cellular Proteins

Poly-Ubiquitin Tag

Ubiquitin

26S Proteasome

Proteasome inhibition results in:
- Impaired cell proliferation
- Activation of apoptotic pathways
- Upregulation of de novo proteasome production

Carfilzomib: A Novel Agent Designed to Promote Selective and Sustained Proteasome Inhibition

- Carfilzomib is a next generation, highly selective, irreversible proteasome inhibitor
  - Potent and sustained target suppression
  - Improved antitumor activity
  - Minimal off-target activity with low neurotoxicity

<table>
<thead>
<tr>
<th>Trial</th>
<th>N</th>
<th>Design/Population</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>003-A0</td>
<td>46</td>
<td>Ph 2 Single-Agent (RR / MM)</td>
<td>Completed</td>
</tr>
<tr>
<td>003-A1</td>
<td>266</td>
<td>Ph 2b Single-Agent (RR / MM)</td>
<td>Completed</td>
</tr>
<tr>
<td>004</td>
<td>165</td>
<td>Ph 2 Single-Agent (R / MM)</td>
<td>Enrolled</td>
</tr>
<tr>
<td>005</td>
<td>50</td>
<td>Ph 2 Single-Agent (RR MM with Renal Impairment)</td>
<td>Enrolled</td>
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<tr>
<td>011</td>
<td>84</td>
<td><strong>FOCUS:</strong> Single Agent vs. Best Supportive Care (R&amp;R MM)</td>
<td>Ongoing</td>
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<tr>
<td>006</td>
<td>84</td>
<td>Ph1/2 Combination with Len/Dex (Relapsed MM)</td>
<td>Enrolled</td>
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<tr>
<td>009</td>
<td>700</td>
<td><strong>ASPIRE:</strong> Combination CRd vs. Rd (Relapsed MM)</td>
<td>Ongoing</td>
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<tr>
<td>007</td>
<td>145</td>
<td>Ph 1b/2 Single-Agent Dose-Escalation (Relapsed Solid Tumors &amp;MM)</td>
<td>Ongoing</td>
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Carfilzomib Active in MM

<table>
<thead>
<tr>
<th></th>
<th>003-A0</th>
<th>003-A1</th>
<th>004 (BTZ-treated)</th>
<th>004 (BTZ-naïve)</th>
<th>006</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>SD</td>
<td>MR</td>
<td>PR</td>
</tr>
<tr>
<td>N</td>
<td>39</td>
<td>257</td>
<td>35%</td>
<td>10%</td>
<td>19%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>41%</td>
<td>12%</td>
<td>9%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>18%</td>
<td>25%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8%</td>
<td>29%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6%</td>
<td>38%</td>
</tr>
<tr>
<td>ORR (≥PR)</td>
<td>18%</td>
<td>25%</td>
<td>21%</td>
<td>54%</td>
<td>78%</td>
</tr>
<tr>
<td>Dosing</td>
<td>20 mg/m²</td>
<td>20/27 mg/m²</td>
<td>20 mg/m²</td>
<td>20/27 mg/m²</td>
<td>15-27 mg/m² + Len (10-25 mg) + Dex (40 mg qw)</td>
</tr>
<tr>
<td># Prior Therapies</td>
<td>5 (median)</td>
<td>5 (median)</td>
<td>1-3 (median)</td>
<td>2 (median)</td>
<td>1-3</td>
</tr>
</tbody>
</table>

Carfilzomib is Well Tolerated as a Single Agent

All treatment-emergent adverse events of Grade $\geq$ 3 ($\geq$10%)

<table>
<thead>
<tr>
<th></th>
<th>003 (A0) (N=46)</th>
<th>003 (A1) (N=266)</th>
<th>004 (N=143)</th>
<th>005 (N=50)</th>
<th>All Studies (N=505)</th>
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<tbody>
<tr>
<td><strong>Hematologic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>26%</td>
<td>27%</td>
<td>15%</td>
<td>20%</td>
<td>23%</td>
</tr>
<tr>
<td>Anemia</td>
<td>37%</td>
<td>22%</td>
<td>12%</td>
<td>28%</td>
<td>21%</td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>28%</td>
<td>19%</td>
<td>12%</td>
<td>18%</td>
<td>18%</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>4.3%</td>
<td>10%</td>
<td>13%</td>
<td>6%</td>
<td>10%</td>
</tr>
<tr>
<td><strong>Non-hematologic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td>11%</td>
<td>8.3%</td>
<td>13%</td>
<td>12%</td>
<td>10%</td>
</tr>
<tr>
<td>Fatigue</td>
<td>8.7%</td>
<td>7.1%</td>
<td>5.6%</td>
<td>14%</td>
<td>7.5%</td>
</tr>
<tr>
<td>Hyponatremia</td>
<td>13%</td>
<td>7.5%</td>
<td>2.1%</td>
<td>4%</td>
<td>6.1%</td>
</tr>
</tbody>
</table>

Two Classes of Proteasomes

Constitutive Proteasome

- β6
- β7
- β1
- β2
- β3
- β4
- β5

Immunoproteasome

- β6
- β7
- LMP2
- LMP7
- MECL1

Unique N-terminal Threonine active sites

Carfilzomib
- 1° Target: chymotrypsin-like active sites (β5 / LMP7);
- 2° Target: LMP2 & MECL1

Bortezomib
- 1° Target: chymotrypsin-like active sites (β5 / LMP7);
- 2° Target: LMP2

Standard Assays for Measuring Proteasome Activity Involve Substrate Cleavage

- Inhibition of both Beta5 & LMP7 necessary to induce Myeloma tumor cell death\(^1\)
  - MM cells express both classes of proteasome

- Substrates for other active sites are poorly defined
  - Role of individual active sites in drug response is currently unknown

**ProCISE**

**Proteasome Constitutive-Immuno Subunit ELISA**

Measures All 6 Proteasome Subunits

1. **Activation**
2. **Probe**

Capture on beads

**ProCISE Measures:**

1. **Active Site Inhibition**
2. **Proteasome Levels**

1. **Wash**
2. **Add 2° Ab**
ProCISE Validation

Recovery Experiment

- Low day-to-day variability
- Beta5 & LMP7 activity is equivalent to LLVY activity
- Dynamic range allows for detection of up to 90% inhibition
Carfilzomib Dosing Schedule & PD Analysis

### QDx2, weekly for 3 weeks (28 day cycle)

<table>
<thead>
<tr>
<th>Time (weeks):</th>
<th>D1</th>
<th>D2</th>
<th>D8</th>
<th>D9</th>
<th>D15</th>
<th>D16</th>
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</thead>
<tbody>
<tr>
<td>% proteasome inhibition</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Rest period (12 d)

### CD138+ (Bone Marrow) vs Blood vs PBMC

<table>
<thead>
<tr>
<th>CD138+ (Bone Marrow)</th>
<th>Blood</th>
<th>PBMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>LLVY</td>
<td>CT-L</td>
<td>CT-L</td>
</tr>
<tr>
<td>ProCISE</td>
<td>Beta5</td>
<td>Beta5</td>
</tr>
<tr>
<td></td>
<td>LMP7</td>
<td>LMP7</td>
</tr>
<tr>
<td></td>
<td>MECL1</td>
<td>MECL1</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th># Patients Analyzed</th>
<th>Blood</th>
<th>PBMC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>74</td>
<td>71</td>
</tr>
<tr>
<td># Patients Analyzed</td>
<td>40</td>
<td></td>
</tr>
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</table>
Kinetics of Proteasome Inhibition in Carfilzomib Treated Patients

- Potent Inhibition After 1st Dose
  - >80% for CT-L

- Cumulative Inhibition in Blood due to irreversible nature of CFZ

- Prolonged Inhibition in PBMCs for 48 hr

- Recovery of activity in PBMC following rest period

Dose: 15 mg/m²
Renal Status or Co-Administration of other Agents Does Not Alter Carfilzomib Pharmacodynamics

PX-171-005
15 mg/m²

PX-171-006

% Activity (Relative to Pre-dose)

LLVY

LMP7

Normal
Mild / Moderate Renal Impairment
Severe Renal Impairment / Dialysis

15 mg/m² CFZ
15 mg/m² CFZ + 40 mg Dex + 10 mg Len
15 mg/m² CFZ + 40 mg Dex + 15 mg Len
High Dose Carfilzomib Results in Near Complete Inhibition of Immunoproteasome Subunits

- Immunoproteasome subunits show dose-dependent inhibition by CFZ
- At 56mg/m², CT-L activity is below the limit of detection (≤10% activity) & total immunoproteasome inhibition reaches 76%
- Only inhibition of Beta5 is seen in Whole Blood
Patient Response May Correlate to Proteasome Subunit Inhibition

Differences in proteasome inhibition are observed between responding & non-responding patients.
## Proteasome Content in Patient-Derived Tumor Cells

### Baseline CD138+ Bone Marrow Proteasome Activity

<table>
<thead>
<tr>
<th>Trial</th>
<th>Ave # Prior Regimens (range)</th>
<th>LLVY (μM AMC / μg Protein) (N = 25)</th>
<th>β5 (ng/μg Protein) (N = 17)</th>
<th>LMP7 (ng/μg Protein) (N = 19)</th>
<th>% LMP7</th>
</tr>
</thead>
<tbody>
<tr>
<td>003, 004, 005</td>
<td>5 (1-13)</td>
<td>10.2 ± 3</td>
<td>0.9 ± 0.1</td>
<td>2.5 ± 0.4</td>
<td>73.7%</td>
</tr>
</tbody>
</table>

### Proteasome Activity in Cell Lines

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>(ng/μg Protein)</th>
<th>LMP7 (ng/μg Protein)</th>
<th>% LMP7</th>
</tr>
</thead>
<tbody>
<tr>
<td>MM1.S</td>
<td>3.9 ± 0.5</td>
<td>2.9 ± 0.2</td>
<td>45%</td>
</tr>
<tr>
<td>8226</td>
<td>4.0 ± 0.5</td>
<td>2.4 ± 0.2</td>
<td>37%</td>
</tr>
<tr>
<td>Arh77</td>
<td>4.0 ± 0.2</td>
<td>3.4 ± 0.2</td>
<td>46%</td>
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</tbody>
</table>

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Carfilzomib Administration Results in Proteasome Inhibition in Tumor Cells

Levels of inhibition similar to Whole Blood & PBMC

2 of 3 patients show inhibition in Bone Marrow Tumor Cells
Comparison of the Proteasome Inhibition Profile of Carfilzomib and Bortezomib

- **LMP2**
  - Pre Post
  - 27 mg/m$^2$ CFZ
  - 1.3 mg/m$^2$ BTZ
  - 20 mg/m$^2$ CFZ

- **MECL1**
  - Pre Post

- **Beta1**
  - 1.3 mg/m$^2$ BTZ
  - 20 mg/m$^2$ CFZ

- **Beta2**
  - 1.3 mg/m$^2$ BTZ
  - 20 mg/m$^2$ CFZ

- **Beta5**
  - 1.3 mg/m$^2$ BTZ
  - 20 mg/m$^2$ CFZ

- **LMP7**
  - Pre Post

- **MECL1**
  - Pre Post

- **LMP2**
  - Pre Post
Conclusions

• ProCISE is the first assay to measure all 6 proteasome subunits in patient samples

• Carfilzomib administration results in ≥ 85% inhibition of CT-L subunits (Beta5 / LMP7)

• Inhibition in MM tumor cells similar to Blood & PBMC

• Levels of inhibition with carfilzomib compare favorably to that of other classes of proteasome inhibitors

• Near complete inhibition of the immunoproteasome is achieved at well-tolerated high-dose CFZ and is currently under further clinical investigation

• Potent immunoproteasome inhibition may correlate with response in MM patients
Acknowledgements

All of the participating research investigators, doctors, nurses and data coordinators

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Gregory Ahmann

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(Biostatistics)
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Lauren Bray
David Eber

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Unit, Kantonsspital, St. Gallen, Switzerland
Christoph Driessen

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