Pediatric Preclinical Testing Program (PPTP) evaluation of the Bcl-2 inhibitor ABT-263

I have no financial relationships to disclose.

- and -

I will not discuss off label use and/or investigational use in my presentation.
ABT-263 Introduction

- Orally bioavailable, BH3 mimetic small molecule inhibitor of Bcl-2 family proteins
- Binds with high affinity to multiple anti-apoptotic Bcl-2 family proteins including Bcl-X_L, Bcl-2 and Bcl-w.
- Like the structurally related compound ABT-737, ABT-263 displays potent cytotoxicity against selected cell lines derived from small cell lung carcinomas and lymphoid malignancies

Pediatric Preclinical Testing Program (PPTP)

- Research contract for testing new agents using *in vitro* and *in vivo* panels of childhood cancers:
  - St. Jude Children’s Research Hospital  Dr. Peter Houghton
  - Children’s Hospital of Philadelphia  Dr. John Maris
  - Albert Einstein College of Medicine  Drs. Richard Gorlick & Andy Kolb
  - Duke University Medical Center  Drs. Steve Keir & Henry Friedman
  - Children’s Cancer Institute Australia  Dr. Richard Lock
  - Children’s Hospital of Los Angeles  Dr. Patrick Reynolds

- *In vivo* panel with 60 xenograft lines & *in vitro* panel with 27 cell lines
- Able to test 10-12 new agents per year against the PPTP childhood cancer panels
Testing of Agents by the PPTP

- **Stage 1 Testing** –
  - Single agent *in vitro* testing
  - *In vivo* efficacy testing at MTD or at dose recommended by sponsor as optimal

- **Stage 2 Testing** – May include one or more of the following:
  - Dose-response for selected sensitive lines
  - Pharmacokinetics
  - Evaluation of target modulation and other pharmacodynamic endpoints
  - Combinations (e.g., with standard chemotherapy agents)
PPTP *In Vitro* Testing

- PPTP *in vitro* panel = 23 lines for Stage 1 testing
- 96 hour exposure at 9 concentrations spanning 4 logs (1.0 nM to 10 μM with replicates of 6 per data point).
- Data analyzed by fitting a non-linear regression sigmoidal dose-response model to the relative fluorescence values vs. the concentration.
PPTP *In Vivo* Testing Procedures – Solid Tumor

- Solid tumor procedures:
  - 10 control & 10 treated mice bearing SC tumors for each xenograft
  - Initiate treatment when the tumors 0.2–0.5 cm$^3$.
  - Tumor diameters measured once weekly intervals with digital vernier calipers.
  - Assuming tumors to be spherical, volumes were calculated from the formula $(\pi/6) \times d^3$, where $d$ represents the mean diameter

- “Event” defined as 4-fold increase in tumor volume

• NOD-SCID mice (8 for treatment group and 8 for control group) inoculated with 3-5 x 10^6 mononuclear cells purified from the spleens of secondary recipient mice.
• Engraftment monitored weekly by flow cytometry, and treatment initiated when the proportion of human CD45^+ cells in the peripheral blood > 1%.
• The proportion of human CD45^+ cells in the peripheral blood monitored weekly throughout the course of treatment.
• Event defined as 25% CD45^+ cells in peripheral blood.

# Objective Response Assessment – Solid Tumors

<table>
<thead>
<tr>
<th>Response</th>
<th>Definition</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progressive Disease 1</td>
<td>&gt;25% increase in tumor volume, and TGD value of ≤1.5</td>
<td>0</td>
</tr>
<tr>
<td>Progressive Disease 2</td>
<td>&gt;25% increase in tumor volume, and TGD value of &gt;1.5</td>
<td>2</td>
</tr>
<tr>
<td>Stable Disease</td>
<td>≤25% increase, and &lt;50% regression</td>
<td>4</td>
</tr>
<tr>
<td>Partial Response</td>
<td>&gt;50% regression</td>
<td>6</td>
</tr>
<tr>
<td>Complete Response (CR)</td>
<td>&lt;0.1cm³ tumor volume</td>
<td>8</td>
</tr>
<tr>
<td>Maintained CR</td>
<td>&lt;0.1cm³ at the end of study</td>
<td>10</td>
</tr>
</tbody>
</table>

*TGD = tumor growth delay*
<table>
<thead>
<tr>
<th>Response</th>
<th>Definition</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progressive Disease 1</td>
<td>CD45% never &lt; 1%, Events before end of study, and TGD* value ( \leq 1.5 )</td>
<td>0</td>
</tr>
<tr>
<td>Progressive Disease 2</td>
<td>CD45% &lt; 1%, Events before end of study, and TGD value &gt;1.5</td>
<td>2</td>
</tr>
<tr>
<td>Stable Disease</td>
<td>CD45% &lt; 1%, and No events before end of study</td>
<td>4</td>
</tr>
<tr>
<td>Partial Response</td>
<td>CD45% &lt;1% for only 1 week</td>
<td>6</td>
</tr>
<tr>
<td>Complete Response (CR)</td>
<td>CD45% &lt;1% for 2 consecutive weeks</td>
<td>8</td>
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<tr>
<td>Maintained CR</td>
<td>CD45% &lt;1% for the last 3 weeks of study</td>
<td>10</td>
</tr>
</tbody>
</table>

*TGD = tumor growth delay*
ABT-263 *In Vitro* Activity

- ABT-263 active against ~ 1/2 of the 23 PPTP cell lines
- EC$_{50}$ values ranged from 0.06 μM for RS4;11 (ALL cell line with MLL gene rearrangement) to > 10 μM for 10 cell lines
- Median EC$_{50}$ for *in vitro* panel was 1.8 μM
- Trend for lower EC$_{50}$ values for the ALL panel compared to the remaining PPTP cell lines (median EC$_{50}$ 0.4 μM vs > 10 μM, p=0.04).
COMPARE-Like Graph for ABT-263

In Vitro Activity

Panel Median EC50 / Line EC50

Ramos (NHL)
Karpas-299 (ALCL)
Kasumi1 (AML)
CCRF-CEM (ALL)
MOLT-4 (ALL)
RS4;11 (ALL)
COG-LL-317 (ALL)
NALM-6 (ALL)
CHLA-136 (NB)
CHLA-90 (NB)
NB-EBC1; p90 (NB)
NB-1643; p40 (NB)
SJ-GBM2 (GBM)
CHLA-258 (EWS)
CHLA-10 (EWS)
CHLA-9 (EWS)
TC-71 (EWS)
CHLA-266 (Rhabdoid)
BT-12 (Rhabdoid)
Rh30 (RMS)
Rh18 (RMS)
Rh41 (RMS)
RD (RMS)
ABT-263 In Vivo Testing Results

- Testing at dose of 100 mg/kg administered orally daily x 21 days
- Solid tumor panel had no objective responses: best response was PD2
- ALL panel had 3 xenografts with CRs and 2 with PD2
- Two T-cell ALL xenografts achieved complete responses that were maintained 3 weeks after the last dose of ABT-263.
Progressive Disease (PD) 1 for ALL-2 and PD2 for ALL-4 and ALL-17

ALL-2

Median %CD45

0 10 20 30 40 50

Time (Days)

Control  Treated

ALL-4

Median %CD45

0 10 20 30 40 50

Time (Days)

Control  Treated

ALL-17

Median %CD45

0 10 20 30 40 50

Time (Days)

Control  Treated
Complete Response (CR) and Maintained CRs for ALL-19, ALL-8 and ALL-16
<table>
<thead>
<tr>
<th>Line</th>
<th>Immunophenotype</th>
<th>Disease Status</th>
<th>CR1 (mos)</th>
<th>Status</th>
</tr>
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<tbody>
<tr>
<td>ALL-2</td>
<td>B-precursor</td>
<td>Relapse</td>
<td>30</td>
<td>DOD</td>
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<tr>
<td>ALL-3</td>
<td>B-precursor</td>
<td>Diagnosis</td>
<td>38</td>
<td>CR2</td>
</tr>
<tr>
<td>ALL-4</td>
<td>B-precursor (Ph+)</td>
<td>Diagnosis</td>
<td>10</td>
<td>DOD</td>
</tr>
<tr>
<td>ALL-7</td>
<td>B-precursor</td>
<td>Diagnosis</td>
<td>7</td>
<td>DOD</td>
</tr>
<tr>
<td>ALL-8</td>
<td>T-cell ALL</td>
<td>Relapse</td>
<td>17</td>
<td>DOD</td>
</tr>
<tr>
<td>ALL-16</td>
<td>T-cell ALL</td>
<td>Diagnosis</td>
<td>120+</td>
<td>CR1</td>
</tr>
<tr>
<td>ALL-17</td>
<td>B-precursor</td>
<td>Diagnosis</td>
<td>25</td>
<td>CR2</td>
</tr>
<tr>
<td>ALL-19</td>
<td>B-precursor</td>
<td>Relapse</td>
<td>4</td>
<td>DOD</td>
</tr>
<tr>
<td>ALL-10</td>
<td>B-precursor</td>
<td>Diagnosis</td>
<td>85+</td>
<td>CR1</td>
</tr>
<tr>
<td>ALL-11</td>
<td>B-precursor</td>
<td>Diagnosis</td>
<td>137+</td>
<td>CR1</td>
</tr>
</tbody>
</table>
Bcl-2 Family Gene Expression –
ALL Panel

- Bcl-2 expression elevated in B-precursor ALL xenografts
- Mcl-1 expression generally low in the ALL xenografts
- Noxa expression high in most ALL xenografts
- ALL-2 only xenograft with no response to ABT-263:
  - Lower expression of Bid and Bim than other ALL xenografts
Conclusions

- ABT-263 shows little single agent *in vivo* activity against the PPTP’s solid tumor panels.
- ABT-263 has remarkable *in vivo* activity against several of the PPTP’s ALL xenografts.
- The single agent anti-leukemia activity observed for ABT-263 supports its rapid clinical evaluation for children with ALL.
- Future preclinical work will explore:
  - Combinations of ABT-263 with standard chemotherapy agents and with molecularly targeted agents that affect apoptosis, and
  - Biological characteristics associated with sensitivity & resistance to ABT-263.
Acknowledgements

- Abbott Laboratories and the ABT-263 team
- NCI research contract NO1CM42216
- Testing teams at each PPTP site