Proteasome inhibitors in cancer chemotherapy

October 15, 2007

Multiple myeloma

Malignancy of differentiated B cells
~14,000 new cases per year in the US
Higher incidence in men and African-Americans
Incurable - Best current therapy extends life expectancy from ~7 months to ~2-5 years
Associated with bone fractures, anemia, renal failure
Tumor cells produce monoclonal immunoglobulins (useful for tracking disease progression)
Tumor cells home to bone marrow (intramedullary disease) and subsequently to other sites (extramedullary)
Multiple myeloma disease progression

MGUS = Monoclonal gammopathy of undetermined significance (1% of adults over 25)

Common mutations in multiple myeloma pathogenesis

Early (primary):
- Cyclin D1, Cyclin D3
- FGFR3 (receptor tyrosine kinase)
  (nearby: MMSET)
- cMAF, MAFB

Late (secondary)
- cMYC
- p53
- Ras family members activated (30%)
- p16 methylation (40%)
- loss of regions of 13q (50%)
Interactions of myeloma cells with other cell types in bone marrow

Normal

Myeloma

![Diagram of interactions]

Bone lesions

![Bone lesions images]
Current treatments for multiple myeloma

Standard: Steroid plus alkylating agent (e.g. prednisone plus melphalan)
Salvage: Vincristine (microtubule depolymerization) plus adriamycin (DNA damage) plus dexamethasone (steroid)
If possible: Autologous bone marrow or stem cell transplant (following high dose chemotherapy and total body irradiation)
   Extends survival 2-5 years
   Alkylating agents must be avoided prior to stem cell harvest

New and experimental treatments

Alpha-interferon - reduces signal transduction via IL-6 (modest effect only)
Thalidomide - inhibits NF-κB signaling, inhibits angiogenesis, alters adhesion of MM to bone marrow stromal cells, other possible mechanisms
   Responses in 1/3 of patients
   Works well in combination with other drugs
   More potent analogs (IMIDs) being developed
Arsenic trioxide - free radical-induced damage, disruption of mitochondrial transmembrane potential
Others include angiogenesis inhibitors, tyrosine kinase inhibitors, proteasome inhibitors
**Bortezomib (Velcade) approved by the FDA in March 2003**

Study in 202 patients with relapsed, refractory myeloma
35% response (extremely high)
Median duration of response: 12 months

Major adverse effect: cumulative peripheral sensory neuropathy

Richardson et al., 2003, NEJM, 348: 26

<table>
<thead>
<tr>
<th>Category of Response</th>
<th>No. of Patients (%)</th>
</tr>
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<tbody>
<tr>
<td>Any response</td>
<td>67 (35)</td>
</tr>
<tr>
<td>Complete or partial response</td>
<td>53 (27)</td>
</tr>
<tr>
<td>Complete or near-complete response</td>
<td>19 (10)</td>
</tr>
<tr>
<td>Partial response</td>
<td>24 (13)</td>
</tr>
<tr>
<td>Minimal response</td>
<td>24 (7)</td>
</tr>
<tr>
<td>No change</td>
<td>44 (24)</td>
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</tbody>
</table>

**Proteasome and ubiquitin**

Threonine protease sites

Aaron Ciechanover, Avram Hersko, Irwin Rose - Nobel Prize 2004
E1 - Ubiquitin activating enzyme (ATP-dependent) only 1 in humans
E2 - Ubiquitin carrying protein
E3 - Ubiquitin-protein ligase many distinct genes responsible for specificity

Proteasome inhibitors

- Olate-lactacyclin-β-lactone
- Danyl-Phenylalanyl-Leucyl-Boronic acid (DPLB)
- Cbz-Leucyl-Leucyl-Leucyl-Methyl vinyl sulfone (Z-LLL-MS)
Proteasome inhibition in live cells:

Up to 80% of cell proteins are processed by the proteasome.
Proteasome inhibition in mammalian cells induces apoptosis and sensitizes cells to pro-apoptotic agents.
Rapidly dividing cells are more sensitive than slowly-dividing cells.

MALIGNANT CELL LINES ARE MUCH MORE SENSITIVE

PS-341
Bortezomib

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p53 and regulation of apoptosis

Mdm2 is an E3 for p53.
p53 half-life normally very short.
DNA damage, etc. will cause p53 phosphorylation, inhibit Mdm2 binding.
High levels of p53 block cell cycle at G1 checkpoint, activate transcription of genes that trigger apoptosis, inhibit transcription of genes that block apoptosis.
p53 status of tumor cells is not well-correlated with susceptibility to PS-341.
Control of NF-κB signaling by the proteasome

Note ubiquitination of TRAF6 with Lys63-linked chains is associated with IKK activation, not degradation.

NF-κB inhibition is not sufficient to explain sensitivity of myeloma cells to proteasome inhibitors

Relative DNA synthesis in three multiple myeloma cell lines

Note: MM cell lines come from very late stage tumors

Hideshima et al., 2002, J. Biol. Chem. 277: 16639-16647
What else might PS-341 be doing?

Mitochondrial injury via Bcl-2 inhibition, generation of reactive oxygen species

Triggering unfolded protein response in the endoplasmic reticulum (note high levels of protein secretion)

Cell-wide depletion of ubiquitin; will affect signaling as well as protein degradation

Effects on histones: Histones are often monoubiquinated, inhibitors of histone deacetylase suppress proteosome activity and sensitize cells to PS-341

Induction of caspase expression (how?)

Down-regulation of E-selectin and VCAM-1 (how?)

Anti-angiogenic (how?)

Pathways induced and repressed by proteasome inhibition in a multiple myeloma cell line

Some strains from the yeast deletion collection show increased sensitivity to proteasome inhibitors

<table>
<thead>
<tr>
<th>Strain</th>
<th>MIB 30 µM</th>
<th>MIB 50 µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>&gt;1,300</td>
<td>1,280</td>
</tr>
</tbody>
</table>

Group 1: Complement proteasome degradation
- |ysd1| 100 | 150 |
- |ype10| 200 | 40 |
- |psa7| 650 | 150 |

Group 2: Mitosis
- |vma10| 250 | 120 |
- |vma23| 800 | 400 |
- |dod4| 1,000 | 200 |

Group 3: DNA repair
- |ops1| 250 | 170 |
- |rea80| 250 | 250 |
- |reaM| 400 | 170 |

Group 4: Nuclear import/morphology
- |mga2| 250 | 200 |
- |neol| 250 | 500 |
- |ynu60W| 1,000 | 200 |

Group 5: General transcription factors
- |mfg2| 130 | 40 |
- |stg3| 130 | 40 |
- |ntl2| 150 | 40 |
- |eas4| 600 | 400 |
- |ynu60W| 1,000 | 200 |
- |yol204w| 1,000 | 600 |
- |gpd5| 200 | 200 |
- |org7| 250 | 250 |
- |org7| 600 | 200 |
- |org7| 1,000 | 40 |

Timing of inhibition in mouse tumors indicates cumulative effects

Ubiquitin-luciferase construct expressed in tumor cells; imaged in living mice

Ub-FL level fails to revert to baseline after 5-7 cycles


Luker et al., 2003, Nature Medicine, 9:969
## Future issues

Will bortezomib be useful for other cancers?

- Ongoing trials for colorectal, pancreatic, liver, lung (non-small-cell), prostate, ovarian, kidney & breast cancers, as single agent or in combinations

What are the best drug combinations?

- Sensitized background screens

Some tumors are resistant; how?

Will other proteasome inhibitors have the same effect?

Can proteasome inhibitors be used as anti-inflammatory drugs?

## Papers for Wednesday
