The Ubiquitin-Proteasome Pathway: Friend and Foe in Multiple Myeloma

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Ubiquitin-Dependent Proteolysis

Target

Ub E1 E2/UBC E3

Target + ATP → Ub E1 E2/UBC E3 → Oligo-Peptides (4-24 residues)

26S

Proteasome Inhibition and Apoptosis

- Proteasome inhibition induces apoptosis preferentially in transformed cells
  - NHL
  - CLL
  - Multiple myeloma


Molecular Consequences

• Accumulation of p53
  – Cell cycle arrest
    • Transactivation of p21
    – Apoptosis through Bax
• Accumulation of Cdkis
  $p21^{Cip1}$ and $p27^{Kip1}$
  – Cycle arrest; apoptosis
  – p21 independent of p53
• Accumulation of Bax
  – Apoptosis

• Activation of JNK
  – Mitochondrial release of cytochrome c and Smac
  – Activates caspase-9
• Up-regulation of TRAIL death receptors 4 and 5
  – Activates caspase-8

Bone Marrow Sampling

- Pre: 41% plasma cells
- Post: 1% plasma cells

## Phase II of Bortezomib in Myeloma

<table>
<thead>
<tr>
<th>Category of Response</th>
<th>No. of Patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 193</td>
<td></td>
</tr>
<tr>
<td>Complete or near-complete</td>
<td>19 (10)</td>
</tr>
<tr>
<td>Immunofixation negative</td>
<td>7 (4)</td>
</tr>
<tr>
<td>Residual immunofixation positive</td>
<td>12 (6)</td>
</tr>
<tr>
<td>Partial response</td>
<td>34 (18)</td>
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<tr>
<td>Complete or partial response</td>
<td>53 (27)</td>
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<tr>
<td>Minimal response</td>
<td>14 (7)</td>
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<tr>
<td>Any response</td>
<td>67 (35)</td>
</tr>
<tr>
<td>Stable disease</td>
<td>46 (24)</td>
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</tbody>
</table>

Challenge I

• While bortezomib was a significant advance, the vast majority of patients had less than a PR
  – 57% (phase III) - 73% (phase II) had ≤ minor response
  – Even those with a PR, near-CR, or CR relapsed

• Many patients could not complete a course of therapy because of drug & dose-related toxicities like peripheral neuropathy
  – Dose reductions in 12%; discontinuations in 18%
Proteasome Inhibition, MKP-1 and JNK

JNK/SAPK MAPK

MEKK1

SEK/MKK4

SAPK/JNK

MKP-1/2

Bortezomib

V PI

MKP-1

HSC-70

Apoptosis

Cytochrome c

Anthracycline + Bortezomib

- Combinations of doxorubicin and bortezomib had a greater-than-additive impact on apoptosis
- Associated with suppression of MKP-1, and heightened JNK activation
- Also seen in vivo

Phase I of Bortezomib + PLD

- TTP was 9.3 mos. vs. 3.8 mos. on the prior therapy – $p = 0.02$
- TTR 24 mos.

DOXIL-MMY-3001 Study

Statistical analysis:
HR (95% CI) 1.82 (1.41-2.35)
p = 0.000004

Why Are Myeloma Cells So Sensitive?

- Plasma cell differentiation reduces proteasome capacity and enhances accumulation of Ub-protein conjugates
- Differentiating 1.29μ+ cells

Proteasome Activity & Sensitivity

• Greater light chain production is associated with a larger load of poly-ubiquitinated proteins

• Greater proteasome activity is associated with a lesser sensitivity to bortezomib

Proteasome Load/Capacity Ratio

The Unfolded Protein Response

Overview

Enhancing factors
- Large protein synthetic rate
- Misfolding protein
- Chaperone capacity
- Inflammation/malignancy
- Proteasome inhibition

Reducing factors
- Deubiquitination
- Aggresome function
- Autophagy
- Oligosecretory escape

Proteasome load
Proteasome capacity
Proteasome inhibition
Apoptotic trigger

Enhancing factors
- Proteasome subunits
- Proteasome assembly
- Proteasome activators

Reducing factors
- Ageing
- Senescence
- Proteasome inhibition

Challenge II

- Combination therapies based on bortezomib show enhanced activity, but also carry with them a risk for increased toxicity.
- Resistance to proteasome inhibition arises whether one uses single-agent bortezomib, or rationally-designed combination regimens.
Modeling Bortezomib Resistance

- Chronic bortezomib exposure produces resistant cells
- No β5 mutations

Irreversible Inhibition & Resistance

• Carfilzomib retains activity against bortezomib-resistant cell line models and primary samples

Phase I of Carfilzomib

- Tolerable and active in daily x2 or x5 dosing

Challenge III

• While carfilzomib was also an advance, it overcomes bortezomib resistance in only a minority of patients

• Mechanisms of resistance to bortezomib in carfilzomib-insensitive patients were unknown, and carfilzomib resistance emerges rapidly as well
Models of Bortezomib Resistance

- In Jurkat T-cell model
- Reduces bortezomib binding
- Confers resistance

GSEA of Bortezomib-resistant Models

Table 2. Significance data for gene set enrichment analysis comparisons between wild-type and BR cells for the myeloma lines studied using sets of IGF-1-regulated genes in MCF-7 breast carcinoma.

<table>
<thead>
<tr>
<th>Gene set</th>
<th>Total no. of genes</th>
<th>ES</th>
<th>NES</th>
<th>Nominal P value</th>
<th>FDR q value</th>
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<tbody>
<tr>
<td>Up in MCF-7 with IGF-1</td>
<td>377</td>
<td>0.45</td>
<td>1.94</td>
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<td>.071</td>
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<tr>
<td>OPM2*</td>
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<td>0.36</td>
<td>1.57</td>
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<tr>
<td>8226</td>
<td></td>
<td>0.38</td>
<td>1.59</td>
<td>.000</td>
<td>.214</td>
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<tr>
<td>ANBL-6</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Down in MCF-7 with IGF-1</td>
<td>374</td>
<td>-0.45</td>
<td>-1.89</td>
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<tr>
<td>OPM2*</td>
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<td>-0.45</td>
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<tr>
<td>8226</td>
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<td>-0.37</td>
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<tr>
<td>ANBL-6</td>
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</table>

ES indicates enrichment score; NES, normalized enrichment score; and FDR, false discovery rate.
*OPM2 was studied twice.

- Dysregulated signaling through IGF-1/IGF-1R identified as prominent

Validation *in vivo*

- In resistant xenograft, OSI-906/bortez → tumor regression
  - Several combo mice had no detectable tumor

• Supports targeting AKT

XBP1 and Resistance

TJP1 Identified as Gene of Interest

- By RNAi, knockdown conferred resistance
- Decreased expression in resistant cells

TJP1 Knockdown Confers Resistance

• Also, over-expression confers sensitivity

TJP1 and MHC Class II

- TJP1 knockdown -> over-expression of class II genes
- Immuno-proteasome subunits in this region

Proteasome Subunits and Class II

- PSMB8 and 9 are in the class II region, and influenced by TJP1.

Targets of TJP1

- STRING analysis and co-IP support an interaction with EGFR and TJP1

Model

Biomarker: Arkansas Data


Biomarker: Millennium Data

P = 0.000246 and 0.00922
Proteasome Maturation Protein

Using ATRA to Suppress NRF2

Challenge IV

• Proteasome inhibition in myeloma therapy is now well established, and additional inhibitors are likely to provide only modest incremental benefits

• Are there new approaches or insights that could sufficiently add to our ability to treat myeloma while leveraging what we know about the ubiquitin-proteasome pathway
Protein Targeting Chimeric Molecules

- Methionine aminopeptidase-2 is degraded by Protac-1, which contains the IκBα phosphopeptide recognized by the F-box protein βTRCP.

BET PROTACs in Cell Lines

BET PROTACs in Other Models


<table>
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<th>MM1.S</th>
<th>U266</th>
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<tbody>
<tr>
<td></td>
<td>TAK-243</td>
<td>Bortezomib</td>
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<tr>
<td>p-PERK</td>
<td>0  5  10  25  50</td>
<td>0  5  10</td>
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<td>ATF6</td>
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<td>XBP1</td>
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<td>β-actin</td>
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<td>BiP</td>
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<td>HSP70</td>
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<td>NRF2</td>
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<tr>
<td>Histone H3</td>
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Overcoming Drug Resistance

Other Areas of Focus

- Novel pathways that confer PI resistance
- Targeting HSP70 to resensitize to PIs and other agents
- Validating additional UPP targets, including E3 ligases
- Pathobiology of high-risk deletion 17p myeloma with loss of TP53
Clinical Conclusions: Friend

- Proteasome inhibitors are especially active drugs against myeloma, likely due to the reliance of plasma cells on the UPR.
- Clinical activity of these agents has contributed to a doubling of the overall survival of myeloma patients seen over the past decade.
- Additional rationally based combinations are emerging that will likely extend these benefits further in all settings of this disease.
Molecular Conclusions: Foe

- Knowledge of the mechanisms of resistance is beginning to emerge.
- These may include decreased immunoglobulin synthesis and/or increased proteasome capacity.
- Both of these (and others) would tip the proteasome load/capacity ratio further away from a state that is sensitive to proteasome inhibitors.
Future Directions

- Validate biomarkers to identify patients most likely to benefit from proteasome inhibitors
- Identify targeted therapies to suppress mechanisms of resistance that will enhance activity in drug-naïve and pre-treated patients
- Use biomarkers to triage patients to the regimens that will most benefit them based on a molecular understanding of their disease clones
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