Oncogenomics to Target the Tumor Cell in its Microenvironment

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Tumor-Promoting Influence of the Microenvironment

- **Growth factors** promote tumor cell proliferation and survival

- **Cytokines and chemotactic factors** promote tumor cell migration and invasion

- **Proteases** break down basement membrane, alter architecture of tissue structures, and promote migration/invasion of tumor cells
Tumor Vasculature-Blood Vessels

- Tumor endothelial cells are uniquely altered in different tumors
- Tumors produce factors (VEGF, FGF) that recruit endothelial cells
- An anti-angiogenesis drug that targets VEGF is FDA approved (Avastin)

Gene-Expression Profiles Associated With Progression to Myeloma

Normal  MGUS  Myeloma

Molecular Pathogenesis of Myeloma

91 GENES
Oncogenes – BCL2, LAF4
Transcription – FOXG1A, RING1
Development – SHH, WNT

22 GENES
Transcription – RING1
Development - FRZB

172 GENES
Membrane – CD38, CD27
Tumour Suppressor – RB, ARMET
Transcription – XBP-1, ZFP
Death – TAX1BP1, TXNL

52 GENES
Survival – TNFSF7
Signalling – MD2, MACS
Structural – ADD1, VCL

ADHESION
DNA
REPAIR

Oncogenomics to Identify Targeted Therapies

Integrated platform aCGH, SKY and expression profiling

55 MM Cell Lines; 73 Patient Samples

46 amplicons - 658 NCBI genes

Expressed Genes: 258

Functional validation of MM candidate genes.

Small molecule

Monoclonal Abs

Anderson and Depinho
Classification Based Upon Expression Profiling

- FGFR3
- MMSET
- C-MAF
- ITGB7
- CCND3
- CCND1
- CCND2

Proliferation
Hyperdiploid

PB, PC, 4p16, maf, 6p21, 11q13, D1, D1+D2, NONE, D2, HMCL

TC Classification

Bergsagel et al. Blood 2005
Individualized Targeted Therapy

- Transformation
  - Differentiation
  - Anti-apoptosis
  - FGFR3 Inhibitor
  - Telomerase
  - Mcl-1 antisense
  - FGFR3
  - Telomerase Inhibitors
    - Antisense
    - Telomestatin
  - FGFR3
  - Xbp-1
  - FLIP
  - CDC34
  - Ribosomal proteins
  - Transcriptional Activity
  - Proteasome
  - Proteasome Inhibitor – PS-341
  - Ubiquitin/proteasome
- Stress Response
  - Anti-angiogenic Agents
  - Geldanamycin
  - Angiogenesis
  - Angiopoetin-1/IL8
  - Hsp70/90

Munshi et al. Blood 2004; 103:1799
Gene Modulations Triggered by Binding of MM Cells to BMSCs
In Vivo Model of Human MM in Human BM Milieu

Bone chip

Human bone chip

Human bone chip

before

after 12 days

B-B4-DM1 (150 μg/Kg)

Tassone P et al. Blood 2005
**MM cells**

- **Cytokines**
  - IL-6, IL-1β, HGF, IL-8, IGF-1, Gas6, MIP2α, -2α, CXCL-1, -5, -6, -10, -13
  - DKK-1, Wnt-5a, SHH

- **Chemokines**
  - FLIP, survivin, cIAP-2, Mcl-1

- **Apoptosis regulation**
  - Hsp90, hsp70

- **Heat shock proteins**
  - 26S proteasome subunits
  - ubiquitin B, UCEs
  - USPs
  - pim-1, pim-2

- **Oncogenes**
  - XBP-1, c-maf, TCF8, rel-B
  - eIF-2α, -3, -4, HDAC1

- **Transcription/translation control**

**BMSCs**

- **Cytokines**
  - IL-6, VEGF
  - IGF-1, LIF

- **Microenvironmental interactions**
  - integrin β5
  - fibrillin 1
  - collagen Vα1

- **Proteasome pathway**
  - 26S subunits
  - 6, -4, ATPase 3, non-ATPases 3, -4, -7

- **Transcription control**
  - HDAC2
Thal/IMiDs Target MM Cells in the BM Microenvironment

A. Thalidomide /IMiD

B. Thalidomide /IMiD

C. Thalidomide/IMiD

Bone Marrow Stromal Cells

IL-6

TNF-α

IL-1β

MBM Vessels

ICAM-1

VEGF

bFGF

CD8+ T Cells

NK Cells

PBMC

D. Thalidomide /IMiD

E. Thalidomide /IMiD

References:

Gupta et al. Leukemia 2001; 15: 1950
Mitsiades et al. Blood 2002; 99: 4525
Mechanisms Whereby IMiDs Augment Anti-MM Immunity

Preclinical (2000): targets tumor (caspase-8 mediated apoptosis) and microenvironment in vitro and in vivo in animal model

- Phase I trial (2001): MTD; favorable toxicity; stable disease or response in 79% patients
- Phase II trials (2002-3): 80% stable disease or response
- Two Phase III trials (2003-4): Revlimid/Dex versus Dex/placebo in relapsed myeloma
Phase III Trials

<table>
<thead>
<tr>
<th>Time to Progression (months)</th>
<th>Proportion of Patients</th>
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</thead>
<tbody>
<tr>
<td>2.5</td>
<td>0</td>
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<tr>
<td>5</td>
<td>0.2</td>
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<tr>
<td>7.5</td>
<td>0.4</td>
</tr>
<tr>
<td>10</td>
<td>0.6</td>
</tr>
<tr>
<td>12.5</td>
<td>0.8</td>
</tr>
<tr>
<td>15</td>
<td>1.0</td>
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<tr>
<td>17.5</td>
<td>0.8</td>
</tr>
<tr>
<td>20</td>
<td>0.6</td>
</tr>
<tr>
<td>22.5</td>
<td>0.4</td>
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</table>

**Phase III Trials**

<table>
<thead>
<tr>
<th>CR (%)</th>
<th>CR+PR (%)</th>
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</thead>
<tbody>
<tr>
<td>Len/Dex</td>
<td>20.2</td>
</tr>
<tr>
<td>Dex</td>
<td>4.0</td>
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</tbody>
</table>

P < .000001
Bortezomib: From Bench to Bedside

1994  NF-κB is a therapeutic target in myeloma
1995-7 Drug discovered (Julian Adams), NCI 60 cell line
1998  Phase I trials started
2000  Phase I trials: safe and has anti-MM activity
2000  Targets MM cell and BM microenvironment to overcome drug resistance in laboratory and animal studies
2001  Phase II trial: 35% responses (including CRs), duration 12 months, with associated clinical benefit shows remarkable responses in patients with advanced disease unresponsive to known therapies
2003  Accelerated approval for relapsed refractory disease by FDA

2003  Phase III trial fully accrued and stopped early due to delay in TTP in Bortezomib cohort

2005  FDA approval extended to relapsed myeloma

2004  Phase II trials upfront and in combination
Combination of Bortezomib + Revlimid

Patient Cells

% cell death

% control

Bortezomib (nM)

0
10
20

0 uM
5 uM

0
10
20
30
40
50

IMID3 (72 h)
PS341 3 nM
IMID3 (72 h)+PS341

Revlimid

0 uM
5 uM
Schema

<table>
<thead>
<tr>
<th>Bortezomib, mg/m²</th>
<th>Lenalidomide, mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Cohort 1</td>
</tr>
<tr>
<td>1.3</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Cohort 2</td>
</tr>
<tr>
<td></td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Cohort 3</td>
</tr>
<tr>
<td></td>
<td>20</td>
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<tr>
<td></td>
<td>Cohort 5</td>
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<tr>
<td></td>
<td>Cohort 6</td>
</tr>
<tr>
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<td>Cohort 7</td>
</tr>
<tr>
<td></td>
<td>Cohort 8</td>
</tr>
</tbody>
</table>

- 8 cohorts of 3–6 pts, with additional 10 pts enrolled at the MTD

21-day cycle*

*For a maximum of 8 cycles; for pts with PD Dex added (20mg day of and day following bortezomib): extension phase for pts in response
## Results

### Baseline Characteristics (N = 12)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
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</thead>
<tbody>
<tr>
<td>Median age, y (range)</td>
<td>59 (37–75)</td>
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<tr>
<td>Male, %</td>
<td>42</td>
</tr>
<tr>
<td>Myeloma type, %</td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>83</td>
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<tr>
<td>IgA</td>
<td>17</td>
</tr>
<tr>
<td>Durie-Salmon stage III, %</td>
<td>25</td>
</tr>
<tr>
<td>Disease status</td>
<td></td>
</tr>
<tr>
<td>Relapsed, n</td>
<td>6</td>
</tr>
<tr>
<td>Relapsed and refractory, n</td>
<td>6</td>
</tr>
<tr>
<td>Median no. of prior therapies (range)</td>
<td>4 (1-9)</td>
</tr>
<tr>
<td>Prior SCT</td>
<td>8 (66%)</td>
</tr>
<tr>
<td>Prior bortezomib</td>
<td>4 (33%)</td>
</tr>
<tr>
<td>Prior lenalidomide</td>
<td>2 (17%)</td>
</tr>
<tr>
<td>Prior thalidomide</td>
<td>9 (63%)</td>
</tr>
<tr>
<td>Cohort</td>
<td>Regimen</td>
</tr>
<tr>
<td>--------</td>
<td>---------</td>
</tr>
<tr>
<td>1</td>
<td>Bortezomib 1.0 mg/m² + lenalidomide 5 mg</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Bortezomib 1.3 mg/m² + lenalidomide 5 mg</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Bortezomib 1.0 mg/m² + lenalidomide 10 mg</td>
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<tr>
<td></td>
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</tr>
<tr>
<td>4</td>
<td>Bortezomib 1.3 mg/m² + lenalidomide 10 mg</td>
</tr>
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</tr>
</tbody>
</table>

*Evaluable  **Dex added

RR (CR + PR + MR) : 91%
Toxicity

• No discontinuations

• No significant PNY, fatigue

• No DLT through first 3 cohorts

• One DLT in cohort 4
  – Grade 3 hyponatremia

• 4 additional pts will be enrolled in cohort 4
  – @ Bortezomib 1.3 mg/m², lenalidomide 10 mg

• Dose reductions of bortezomib (1.3 to 1.0 mg/m²) (n=4), lenalidomide (10 to 5 mg) (n=1) beyond cycle 2 (neutropenia, thrombocytopenia)

• MTD not yet reached
Based upon gene profiling:
Bortezomib and Hsp90 inhibitor 17 AAG

Based upon cell signaling:
Bortezomib and HDAC6 inhibitor tubacin

Based upon correlative science:
Bortezomib and p38 MAPK inhibitor SCIO 469
Proteasome Inhibition by Bortezomib

- Bortezomib inhibits the proteasome, leading to accumulation of unfolded proteins.
- Unfolded proteins bind to heat shock proteins (HSPs).
- HSPs aggregate proteins into aggresomes.
- Aggresomes are internalized and degraded by lysosomes.

Key terms:
- Proteasome
- HSP
- Aggresome
- Microtubule
- Lysosomal degradation
Therapeutic Value of HSP inhibition Following Bortezomib Treatment

- Control
- Bortezomib
- 17AAG
- Bortezomib + 17AAG

% Cell death
Therapeutic value of Aggresome Inhibition by Tubacin

Dynein

Microtubule

Tubacin

AGGRESOME

Proliferation (% control)

Tubacin (µM)

MM cell lines

0 1.25 2.5 5 10 20

160

120

80

40
Synergistic Anti-Proliferative Activity of Bortezomib and Tubacin

Tubacin

BORTEZOMIB

AGGRESOME

PROTEASOME

Proliferation (% control)

0 5 10

0 5 10 nM

Tubacin (µM)
Gene Microarray Predicts Clinical Response to Proteasome Inhibitor

- Fc alpha receptor
- Hrk activator of apoptosis
- Hsp 27
- N-ras
- Programmed cell death 10
- Cancer/testis antigen 2
- Ubiquitin carrier protein
p38 MAPK Inhibitor (SCIO-469) Enhances Bortezomib-Induced Cytotoxicity

Hideshima et al. Oncogene 2004; in press
Conclusions

1. A new treatment paradigm targeting both the tumor cell and its microenvironment can overcome drug resistance.

2. Ongoing oncogenomic and proteomic studies are defining novel targets governing tumor cell and host interactions as well as informing clinical protocol design.