Translation from Preclinical Model Systems to the Bedside in Multiple Myeloma

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Harvard Medical School
Cancer Drug Development (2005)
14 New Drugs in 2004, Cost 40 billion

Preclinical Cancer Drug Candidates

5% Success To Market in 7-10 years
>60% Fail Due To Lack of Efficacy

Effective FDA-approved Cancer Drug

<table>
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<th>Pre-clinical testing</th>
<th>Oncology compounds</th>
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<td>Approval</td>
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Challenges in Drug Development

- Cancer Gene Discovery
- Mechanism of Action
- Clinico-Pathologic Studies
- Cancer Biology

High-Throughput Assays
Drug Discovery
Preclinical Testing

Clinical Trials

ACADEMIA
BASIC & TRANSLATIONAL

INDUSTRY

ACADEMIA
CLINICAL

14 New Drugs in 2004, Cost 40 billion
Chromosomes and Prognosis in Multiple Myeloma

Incurable plasma cell dyscrasia with conventional and high dose therapy

Novel treatment approaches can overcome high risk cytogenetic abnormalities t(4;14), del(17p), del(13q14), i.e. bortezomib, lenalidomide

Future Directions:
Oncogenomics to identify novel targets, improve patient classification, and inform design of combination clinical trials
Classification Based upon Expression Profiling

FGFR3  MMSET  C-MAF  ITGB7  CCND3  CCND1  CCND2

Proliferation

Hyperdiploid

Classification Based upon Expression Profiling

Bergsagel et al, Blood 2005
Oncogenomics to Identify Targeted Therapies

Integrated platform aCGH, SKY and expression profiling

55 MM Cell Lines; 73 Patient Samples

Expressed Genes: 258

Functional validation of MM candidate genes.

Small molecule


Monoclonal Abs Vaccines
Multiple Myeloma array CGH prognostic classification

Gene Modulations Triggered by Binding of MM Cells to BMSCs

- Growth
- Survival
- Drug resistance

Adhesion molecules

Cytokines
BM Microenvironment Triggers Proteasome Activity in MM Cells

Chauhan et. al., 2006
Plasmacytoid Dendritic Cells Promote the Growth of MM Cells Ex-Vivo

Myeloma Patient

Healthy donor or Myeloma patient

CD138+ MM cells

Plasmacytoid dendritic cells

Co-culture

Proliferation/Propagation of patient MM cells

Separation of MM cells with CD138-magnetic beads for further utilization

* 4-5 fold increase in growth of MM cells
* Increase in IL-1-α, IL-6, TNF-α & VEGF
* NF-κB induction

Potential use for deriving new MM cell lines

In Vitro studies of drug-efficacy/drug-resistance

Genomics & Proteomic studies

* 4-5 fold increase in growth of MM cells
* Increase in IL-1-α, IL-6, TNF-α & VEGF
* NF-κB induction

Chauhan et al., 2006
In vivo Model of Human MM in Human BM Milieu

Tassone P. et al., Blood, 2005
XBP-1s Transgenic Mice

Carrasco et al, 2006
Novel Therapies Targeting Myeloma Cells in the BM Microenvironment

**Targeting MM Cell**
- IGF-1 inhibitors, CD40 Ab
- 17-AAG, PK11195, Smac mimetics
- Telomestatin, CHIR 258, Rad 001

**BM Microenvironment**

- **BM**
- stromal cells
- **Targeting MM Cell and BM Milieu**
  - Bortezomib, NPI0052
  - Thalidomide, Revlimid
  - SAHA, Tubacin
  - PTK787, Perifosine

**Targeting BM Milieu**
- IKK inhibitors, defibrotide
- p38MAPK inhibitors
Integration of Novel Therapy Into Myeloma Management

Bortezomib, Lenalidomide, Thalidomide, Doxil (FDA Approved Since 2003)

Treatment of Relapsed/Refractory MM (single agent/combinations)

Induction/First-line Therapy

Transplant/Maintenance
Bortezomib Targets MM Cells in the BM Microenvironment

- Bortezomib inhibits MM cell growth.
- Bortezomib reduces cytokine production (IL-6, TNFα).
- Bortezomib targets bone marrow stromal cells, decreasing ICAM-1 and VCAM-1 expression.
- Bortezomib inhibits VEGF and bFGF production.

References:
Bench to Bedside Development of Bortezomib in Myeloma

<table>
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<tr>
<th>Year</th>
<th>Event</th>
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<tbody>
<tr>
<td>2000</td>
<td>Phase I trials: safe and has anti-MM activity</td>
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<tr>
<td>2000</td>
<td>Targets MM cell and BM microenvironment to overcome drug resistance in vitro and in vivo</td>
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<tr>
<td>2001</td>
<td>Phase II trial in relapsed refractory MM</td>
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<tr>
<td>2003</td>
<td>FDA approved: 35% responses (CRs), duration 12 months, clinical benefit</td>
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<td>2004</td>
<td>Phase III trial versus Dex in relapsed MM</td>
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<tr>
<td>2005</td>
<td>FDA approved: prolonged TTP and OS</td>
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<td>2006</td>
<td>High OR and CR rates as initial therapy</td>
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<td>2006</td>
<td>Novel proteasome inhibitors and combinations</td>
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## Bortezomib in Newly Diagnosed Multiple Myeloma

<table>
<thead>
<tr>
<th>Study</th>
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<th>N</th>
<th>CR/nCR</th>
<th>ORR (CR+ PR)</th>
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<tr>
<td>Mateos (<em>Blood</em> 2006)</td>
<td>Bz + MP</td>
<td>53</td>
<td>43%*</td>
<td>89%</td>
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<tr>
<td>Jakubowiak (ASH 2006)</td>
<td>Bz + Doxil + Dex</td>
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<td>32%</td>
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<td>Wang (ASH 2005)</td>
<td>Bz -TD</td>
<td>38</td>
<td>18%</td>
<td>92%</td>
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<td>Oakervee (<em>BJH</em> 2005)</td>
<td>PAD 1(^{st}) cohort (1.3)</td>
<td>21</td>
<td>29%</td>
<td>95%</td>
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<td>Popat (ASH 2005)</td>
<td>PAD 2(^{nd}) cohort (1.0)</td>
<td>19</td>
<td>16%</td>
<td>89%</td>
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<td>Badros (ASH 2005)</td>
<td>Bz +DT-PACE</td>
<td>12</td>
<td>17%</td>
<td>83%</td>
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<tr>
<td>Barlogie (ASCO 2006)</td>
<td>Bz+ DT-PACE</td>
<td>249</td>
<td>80%**</td>
<td>92%**</td>
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</table>
Bortezomib Effects on Myeloma Cells

1. Downregulates adhesion molecules, caspase dependent cleavage of IL-6R
2. Activates JNK, increased ROS, mitochondrial release of cyto C/Smac, caspase 9>8, 3 cleavage
3. Induces p53+/- apoptosis
4. Induces cleavage of Mcl-1
5. Inhibits DNA repair (ATM, DNA PKcs cleavage)
6. Induces apoptosis of endothelial cells, osteoclasts
7. Induces osteoblasts and new bone formation
8. Differential effects on proteasome activities
Novel Proteasome Inhibitor NPI-0052 Inhibits Human MM Cell Growth and Prolongs Survival in a Murine Model

Phase I clinical trial in myeloma in MMRC

Chauhan et al, Cancer Cell, 2005.
Lenalidomide

- More potent immunomodulator than thalidomide
  - Up to 50,000 times more potent inhibitor of TNFα
  - 200- to 1000-fold in cytokine modulation
  - Increased stimulation of T-cell proliferation
  - Augmented stimulation of IL-2 and IFNγ production

- Different side effects: no significant constipation, neuropathy, or sedation but more myelosuppressive

- Not teratogenic in New Zealand Rabbit Model

- Evaluated in CLL, AML, Non-Hodgkin’s lymphoma, PTCL, Myelofibrosis, MDS, Waldenstrom’s Macroglobulinemia and Multiple myeloma

Lenalidomide in Myeloma

- MM cells
- Bone Marrow Stromal Cells
- Dendritic Cells
- IL-6
- TNFα
- IL-1β
- IL-2
- IFNγ
- CD8+ T Cells
- Bone Marrow Vessels
- ICAM-1
- VEGF
- bFGF
- NK Cells
- PKCζ
- NFAT
- PI3K
- CD28

References:
Bench to Bedside Development Of Lenalidomide

- Preclinical (2000): targets tumor (caspase-8 mediated apoptosis) and microenvironment in vitro and in vivo in animal model
- Phase I trial (25 patients, 2001): MTD 25 mg; favorable toxicity; stable disease or response in 79% patients
- Phase II trials (324 patients, 2002-3): confirmed responses and decreased neuropathy, constipation, and somnolence compared to thalidomide; Dex improved responses
- Phase II trial (34 patients, 2005) 91% responses, with 6% CR and 32% nCR as initial therapy for transplant candidates; MPR promising for non-transplant candidates.
Objective response defined as: ≥50% reduction in serum M protein and ≥90% reduction in urine M protein, or reduction to <200 mg/24 hr. Responses confirmed by two consecutive determinations at least 4 weeks apart.

Growth of the MM Cell in the BM Microenvironment

Cell surface targets
- CD40
- FGFR3
- CS1
- BAFF-R
- VEGFR

Cytokines
- IL-6, VEGF
- IGF-1, SDF-1α
- BAFF, APRIL, BSF-3
- TNFα
- TGFβ
- VEGF

Cytokines
- Smad, ERK
- NF-κB

Adhesion
- LFA-1
- MUC-1
- VCAM-1
- Fibronectin
- VLA-4

BMSC
- NF-κB

Migration
- GSK-3β
- FKHR
- Caspase-9
- NF-κB
- mTOR
- Bad

Survival
- Anti-apoptosis
- Cell cycle

Proliferation
- Bcl-xL
- Mcl-1

Survival
- Anti-apoptosis
- Cell cycle

Protection
- Akt
- PI3-K
- MEK/ERK

Anti-apoptosis
- NF-κB
- Bcl-xL
- IAP
- Cyclin-D

Molecular Networks
- JAK/STAT3
- p27Kip1

MEK Inhibitor AZD6244 Specifically Inhibits pERK and Induces Apoptosis in MM Cells

A

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B

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<td>INA-6</td>
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<tr>
<td>MCCAR</td>
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<tr>
<td>MM1S</td>
<td>24.86 ± 0.98</td>
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<tr>
<td>MM1R</td>
<td>25.312 ± 1.23</td>
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<td>RPMI8226</td>
<td>13.9 ± 1.03</td>
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<td>DOX40</td>
<td>16.74 ± 2.05</td>
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<td>LR5</td>
<td>7.5 ± 0.945</td>
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<tr>
<td>28PE</td>
<td>28.7 ± 1.368</td>
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<tr>
<td>28BM</td>
<td>0.92 ± 0.034</td>
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C

Patient MM cells

% survival

0 20 40 60 80 100 120

0 0.02 0.2 2 20

AZD6244, μM

Tai ASH # 3460 and 3463, 2006
AZD6244 Blocks Osteoclast Differentiation

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<tr>
<th>AZD6244</th>
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<th>pERK</th>
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B

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C

control  AZD6244

OCL  OCL  OCL

OCL precursor  Bone resorption  Mature OCL

Tai #3460 ASH2006
Rationally Based Combination Therapies

**Based upon gene profiling:**
Bortezomib and Hsp90 inhibitor

**Based upon cell signaling:**
Bortezomib and Lenalidomide
Bortezomib and NPI-0052
Bortezomib and Akt inhibitor
Bortezomib and HDAC6 inhibitor
Bortezomib and Smac peptides
Bortezomib and Bcl 2 inhibitors
Lenalidomide and mTOR inhibitor
Lenalidomide and Anti-CD40 antibody

**Based upon correlative science:**
Bortezomib and p38 MAPK inhibitor
Superior Time to Progression with Combination of Bortezomib and PLD

PLD + Bortezomib 9.3 months

Bortezomib 6.5 months

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

Harousseau et al Abstract 8002
Bortezomib and Hsp 90 Inhibitor Therapy

Hsp 90 gene and protein overexpressed in MM; Bortezomib further upregulates hsp 90 (2002)

Hsp90 inhibitor and Bortezomib induces synergistic cytotoxicity and overcome Bortezomib resistance in vitro and in vivo (2003-4)

Phase I/II clinical trials show safety and that hsp90 inhibitor can sensitize or overcome resistance to Bortezomib (2005-6) (Richardson et al, ASH 2006)

Phase III trial of Bortezomib/hsp90 inhibitor versus Bortezomib in relapsed MM for FDA approval
Bortezomib and Lenalidomide Therapy

Lenalidomide induces caspase 8 mediated apoptosis of MM cells in BM in vitro and in vivo; Dex (caspase 9) enhances response (2000)

Phase I-III clinical trials show favorable toxicity, remarkable activity, and that lenalidomide/Dex prolong OR, CR, TTP, OS versus Dex in relapsed MM leading to FDA approval (2006)

Synergistic MM cell toxicity of lenalidomide with Bortezomib in vitro and in vivo (dual apoptotic signaling)

Phase I-II trials show that majority of patients refractory to either agent alone respond to the combination.

Richardson et al, ASH 2006
Blockade of Ubiquinated Protein Catabolism

Broad

Trial of LBH alone and with Bortezomib

Hideshima et al, Clin Cancer Res;2005; 11: 8530
Compartment-specific bioluminescence imaging (CS-BLI)
High-throughput screening of MM with BMSCs (SPORE to CACS)

McMillin et al. Oral presentation, IMW 2007 abs # S11
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 to enhance cytotoxicity, overcome drug resistance, and improve patient outcome.
Ongoing MM-SPORE Collaborative Model for Rapid Translation from Bench to Bedside

Four new FDA approved drugs in last three years

Pharmaceuticals

DFCI - MM SPORE

Academia

Advocacy MMRF

NIH NCI

FDA