Understanding Biological Activity to Inform Drug Development in Multiple Myeloma

Kenneth C. Anderson, M.D.

Jerome Lipper Multiple Myeloma Center
Dana-Farber Cancer Institute
Harvard Medical School
Targeting Growth of MM in the BM Microenvironment

Hideshima T and Anderson KC. Nat Rev Cancer 2007,
Integration of Novel Therapy Into Myeloma Management

Proteasome inhibitors: Bortezomib, carfilzomib, ixazomib; immunomodulatory drugs: thalidomide, lenalidomide, pomalidomide; HDAC inhibitor: panobinostat; monoclonal antibodies: elotuzumab and daratumumab

Target MM in the BM microenvironment, alone and in combination, to overcome conventional drug resistance in vitro and in vivo

Effective in relapsed/refractory, relapsed, induction, consolidation, and maintenance therapy

18 FDA approvals and median patient survival prolonged 3-4 fold
Bench to Bedside Translation of Novel Agents in Myeloma

Preclinical and Clinical Studies leading to FDA Approvals in MM

- 2006 Thalidomide
- 2012, 2015 Carfilzomib
- 2015 Panobinostat
- 2003, 2005, 2008 Bortezomib (BTZ)
- 2007 Doxil + BTZ
- 2013, 2015 Pomalidomide
- 2006, 2014 Lenalidomide
- 2015 Ixazomib
- 2015 Daratumumab
- 2015 Elotuzumab

Improvement in overall survival from median of 3 to 8-10 years

- 1960-65
- 1965-70
- 1970-75
- 1975-80
- 1980-85
- 1985-90
- 1990-95
- 1995-00
- 2000-05
- 2005-10

Immunomodulatory agent
Proteasome inhibitor
Monoclonal Antibody
HDAC inhibitor
Myeloma Model to Link Partners to Overcome Obstacles in Traditional 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
Multiple Myeloma Collaborative Model for Bench to Bedside Research

- Patients
- Biotech, Pharma
- Academia
- Advocacy
  - MMRF
  - IMF
  - LLS
- NIH
- NCI
- FDA
Immunomodulatory Drugs in Myeloma

**A**
- MM cells
- ICAM-1
- VEGF, bFGF

**B**
- Bone Marrow Stromal Cells
- IL-6 ↑
- TNFα ↑
- IL-1β ↑

**C**
- CD8+ T Cells
- NK Cells
- NK-T Cells

**D**
- Dendritic Cells
- CD28

**E**
- NFAT
- PKCζ
- PI3K

**References**
Mechanism of Action of Immunomodulatory Drugs

Degronimids: Link to ubiquitin 3 ligase complexes

Kronke et al, Science, 2014

Lu et al, Science, 2014
Degronimids Trigger Degradation of Selective Substrates

Target Protein

Ubiquitin Tag

Binding "Warhead"

DEGRONIMID TM

Cereblon Linker

Cereblon E3 Ligase Complex

Ubiquitin Tag

Ubiquitin Tag

Proteasome "Destroy"

Degrade

Recycle Degradation Machinery

Bradner et al, Science, 2015
Proteasome: Present and Future Therapies

Ubiquitin Proteasome Receptor Deubiquitylating Enzymes (DUBs)

P5091 target USP-7

Ub

Poly-ubiquitinated proteins (proteasome substrates)

20S

α

β

19S

Free Ub for re-cycling

Degraded protein

ATPases/Cdc48

Six Protease activities

β5, β5i

β1, β1i

β2, β2i

Potential Therapeutic Targets

Immunoproteasome

26S PROTEASOME

Potential Therapeutic Targets

ATP → ADP

PR-924

β5

20S

Bortezomib, Carfilzomib, CEP-18770 ONYX-0912 Ixazomib Marizomib: β5, β1, β2

PR-924

UB enzymes E1, E2 and E3-UB-Ligases

Six Protease activities

β5, β5i

β1, β1i

β2, β2i

Poly-ubiquitinated proteins (proteasome substrates)

P5091 target USP-7

Ub

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Degraded protein

26S PROTEASOME
Mechanisms Mediating Anti-MM Activity of Bortezomib

**ER-Stress Induction**
- Caspase-12 cleavage;
- ↑ phospo-PERK;
- ↑ GADD-153, ATF4, GRP 78, & XBP-1 splicing

**Anti-angiogenic & Anti-Osteoclastic Activity**
- ↓ Migration, VEGF, Proangiogenic MMP-9, & Caveolin-1;
- ↓ Osteoclastogenesis via MIP1α, BAFF
- ↑ Osteoblast formation

**Apoptosis**
- ↑ JNK; Caspases & PARP cleavage;
- ↑ ROS; ↓ ΔΨm
- ↑ Cyto-c & Smac release; ↓ IAPs;
- ↑ mitochondrial Ca^2+ influx;
- ↑ Bid cleavage, Fas & FasL, BH-3 only proteins: Bim, Bik, & NOXA

**Growth & Survival**
- ↓ NF-κB, MAPK, JAK/STAT
- ↓ IGF-1/IL-6, ↑ PI3K-Akt

**Microenvironment**
- ↓ MM-BMSC’s interaction;
- ↓ ICAM, VCAM, αVβ3
- ↓ IGF-1, IL-6, BAFF, RANKL

**Heat Shock Proteins & DNA Repair**
- ↑ Heat Shock Proteins-27, -70, 90; ↓ DNA-PK

**Cell-Cycle**
- Cdk inhibitors: ↑ P21 & p27, p53
- Cyclins: D1, E1, A, B.

**Proteasome**
- ↓ Chymotrypsin- and Caspase-like proteasome activities;
- ↑ Mono-ubiquitination;
- ↑ 26S Proteasome subunits
Development of Rationally-Based Combination Therapies (HDAC and Proteasome Inhibitors)

Protein → Protein aggregates (toxic) → Ub → 26S proteasome → Bortezomib, Carfilzomib, NPI0052, MLN9708, ONX 0912

Panbinostat, Vorinostat, ACY1215, HDAC6

dynein → Microtubule

HDAC6

Aggresome → Lysosome → Autophagy

Panobinostat or Placebo Plus Bort/ Dex in Relapsed or Relapsed/ Refractory MM

Improvement in median PFS of 4 mos w/o difference in ORR or OS

Two-fold increase in nCR/CR rate (28% vs 16%)

Higher rate of Grade 3/4 diarrhea (25.5% vs 8%), fatigue (23.0% vs 11.9%), thrombocytopenia (67.4% vs 31.4%), and leucopenia (34.5% vs 11.4%), discontinuation due to AE (33.6% vs 17.3%).

FDA approved for relapsed refractory MM exposed to bortezomib and IMiD

Need for less toxic more selective HDACi that can be given with PI to exploit synergistic cytotoxicity, ACY 241.

MAb Based Therapeutic Targeting of MM

**Antibody-dependent Cellular Cytotoxicity (ADCC)**
- Effector cells: NK cell, macrophage, neutrophil...
- Lucatumumab or Dacetuzumab (CD40)
- Elotuzumab (CS1)
- Daratumumab (CD38)
- XmAb®5592 (HM1.24)
- SAR650984 (CD38)

**Complement-dependent Cytotoxicity (CDC)**
- CDC
- MM
- Daratumumab (CD38)
- SAR650984 (CD38)

**Apoptosis/growth arrest via intracellular signaling pathways**
- huN901-DM1* (CD56)
- nBT062-maytansinoid/DM4* (CD138)
- 1339 (IL-6)
- BHQ880 (DKK)
- RAP-011 (activin A)
- Daratumumab (CD38)
- SAR650984 (CD38)
- J6M0-MMAF* (BCMA)

* Ab drug conjugate

Updated from Tai & Anderson Bone Marrow Research 2011
Iterative Bedside to Bench and Back Development of Elotuzumab and Lenalidomide Dexamethasone

- SLAMF7 (CS1) is highly and uniformly expressed at gene and protein level on patient MM and NK cells
- Elotuzumab (Elo) is a humanized monoclonal antibody targeting CS1, activates NK cells via CD 16 and ADCC
- Clinical trial of Elo in MM achieved stable disease
- ADCC activity of Elo against MM enhanced by lenalidomide (len) in preclinical models (Tai et al, Blood 2008)
- Phase II trial: 92% response to len dex elo in relapsed MM, PFS 32.5 months
- Phase III trial shows len dex elo prolongs PFS in relapsed MM by 5 months compared to len dex, leading to FDA approval
Daratumumab: Mechanism of Action

- Human CD38 IgGκ monoclonal antibody
- Direct and indirect anti-myeloma activity\(^1\)\(^-\)\(^5\)
- Depletes CD38\(^+\) immunosuppressive regulatory cells\(^5\)
- Promotes T-cell expansion and activation\(^5\)

Dara, Len, and Dex (DRd) Versus Len and Dex (Rd) in RR MM

- Daratumumab-Rd significantly improved PFS in comparison with Rd alone: 63% reduction in progression/death

- DRd doubled CR/sCR rates and quadrupled MRD-negative rates, with safety profile of daratumumab or Rd alone

  Dimopoulos et al, EHA 2016, NEJM 2016

Dara, Bort and Dex (DVd) versus Bort and Dex (Vd) in R/R MM

Daratumumab-Vd significantly improved PFS, TTP, and ORR in comparison with Vd alone: 61% reduction in progression/death

Daratumumab-Vd doubled VGPR and CR rates, w/o toxicities

  Palumbo et al, ASCO 2016, NEJM 2016
Combinations in the Upfront Treatment of MM

Stewart AK, Richardson PG, San Miguel JF Blood 2009
Is Early Transplant Needed? IFM/DFCI 2009 (N=1,360)

- **Randomize**
  - **Induction**
    - **MRD**
    - **Collection**
      - **Consolidation**
        - **MRD**
        - **Maintenance**
          - **MRD**

**Induction**
- RVD x 3
- CY (3g/m2) MOBILIZATION
  - Goal: 5 x10^6 cells/kg
- Melphalan 200mg/m^2* + ASCT
- RVD x 2
- Lenalidomide*
  
**Consolidation**

**Maintenance**
- Lenalidomide*
- SCT at relapse

**Collection**
- **MRD**
- CY (3g/m2) MOBILIZATION
  - Goal: 5 x10^6 cells/kg
- RVD x 5

**SCT at relapse**

**Randomize**
- **Induction**
- **MRD**
- **Collection**
- **Consolidation**
- **Maintenance**
- **MRD**

**Calibration**
- **RVD x 3**
- **CY (3g/m2) MOBILIZATION**
  - Goal: 5 x10^6 cells/kg

*IFM vs. US: 1yr vs. Continuous

Richardson et al, ASH 2014
## IFM: RVD and Early vs Late ASCT

<table>
<thead>
<tr>
<th></th>
<th>RVD arm N=350</th>
<th>Transplant arm N=350</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CR</strong></td>
<td>49%</td>
<td>59%</td>
<td></td>
</tr>
<tr>
<td><strong>VGPR</strong></td>
<td>29%</td>
<td>29%</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>PR</strong></td>
<td>20%</td>
<td>11%</td>
<td></td>
</tr>
<tr>
<td>&lt;PR</td>
<td>2%</td>
<td>1%</td>
<td></td>
</tr>
<tr>
<td><strong>At least VGPR</strong></td>
<td>78%</td>
<td>88%</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Neg MRD by FCM, n (%)</strong></td>
<td>228 (65%)</td>
<td>280 (80%)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Attal et al, ASH 2015
Achilles Heals: Hallmarks of the Disease that are Vulnerabilities

Excess Protein Production:
Target protein degradation
Trigger selective protein degradation

Immune Suppression:
Restore anti-MM immunity

Genomic abnormalities:
Target and overcome genomic abnormalities
Proteasome: Present and Future Therapies

Potential Therapeutic Targets

ATPases/Cdc48

Immunoproteasome

Bortezomib, Carfilzomib, CEP-18770, ONYX-0912, Ixazomib, Marizomib: β5, β1, β2

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P5091 target USP-7

Ubiquitin Proteasome Receptor

Deubiquitylating Enzymes (DUBs)
USP 7 (DUB) Inhibitor P5091 Overcomes Bortezomib-Resistance in MM

b-AP15, a Novel USP14/UCHL5 Inhibitor, Induces Polyubiquitination Without Blocking Proteasome Catalytic Activities

Clinical Trial Ongoing

Tian et al. Blood 2014; 123: 706-16
Immune Suppressive Microenvironment in MM

- pDC
- MDSC
- TAM
- IL-6, IL-10, TGFβ, PGE, ARG1, NO, ROS, COX2
- Depletion of cysteine

Tumor promotion and induction of PD-L1 expression

- Tregs
- PD1
- CD8
- NKT
- CD4
- NK
- B

Stroma

Checkpoint Blockade Induces Effector Cell Mediated MM Cytotoxicity

Effector: Autologous effector cells (CD3T cells, NK cells)

Target: CD138+ MM cells from Rel/Ref MM-BM

* p<0.05

Lenalidomide with Checkpoint Blockade Reverses MDSC Induced Immune Suppression in MM

Autologous effector cells cultured with MDSC of RR-MM bone marrow

Enhanced Activity of Combination Immune Therapies

WGS at Diagnosis

PD26419c

- 5286 substitutions
- 51 deletions and insertions
- 49 rearrangements
  - t. duplication
  - deletion
  - inversion
  - translocation

Copy number
- LOH
- gain
Venetoclax Therapy of Relapsed/Refractory MM

Figure 4. Objective Response Rates by t(11;14) Status

- All Patients (n=66):
  - ORR 21%: 12%
  - CR: 2%
  - VGPR: 2%
  - PR: 6%

- t(11;14) (n=30):
  - ORR 40%: 20%
  - CR: 3%
  - VGPR: 13%
  - PR: 6%

- No t(11;14) (n=36):
  - ORR 6%: 6%
Achilles Heal: Low YAP1 Expression in Subsets of Hematological Malignancies

Cottini et al Nat Med 2014;20:599-606
Damaged DNA

ATM

Nuclear ABL1

p73

Ub

STK4

Survival

Apoptosis

Damaged DNA

ATM

Nuclear ABL1

p73

Ub

YAP1

Pro-apoptotic genes, cell cycle genes.

Cottini et al Nat Med 2014;20:599-606
Model of KDM3A-KLF2-IRF4 Axis in MM cells

KDM3A catalyses removal of H3K9 mono- and di-methylation in MM

Ohguchi et al Nat Comm 2016
Summary and Future Directions

Discovery and validation of novel agents, alone and in combination, which can overcome conventional drug resistance using in vivo and in vitro models of myeloma in its bone marrow microenvironment.

Clinical trials informed by biomarkers and combinations defined in preclinical studies.

Collaborative effort of academia, biotech/pharma, NIH/NCI, FDA, and advocacy.

Promising future translational efforts target Achilles heels: targeting protein homeostasis, restoring host anti-myeloma immunity, and targeting genomic abnormalities.