Bench to Bedside Translation of Targeted and Immune Therapies in Multiple Myeloma

COMy Lifetime Achievement Award

Kenneth C. Anderson, M.D.

Jerome Lipper Multiple Myeloma Center
Dana-Farber Cancer Institute
Harvard Medical School
Conflict of Interest

Advisor
- Takeda
- BMS
- Giliad

Scientific Founder
- Oncopep
- C4 Therapeutics
Make Science Count for Patients
Treat Patients as Family (Hopkins 1973-80)
### 1980s: Characterization of New Lineage Reactive MoAbs (DFCI)

<table>
<thead>
<tr>
<th></th>
<th>Old Criteria</th>
<th>New Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre-B cell</strong></td>
<td>cyto $\mu$</td>
<td>$B4$ (CD19)*</td>
</tr>
<tr>
<td><strong>B cell</strong></td>
<td>surface Ig</td>
<td>$B1^<em>$, $B2$ (CD21)</em>, $B5^*$</td>
</tr>
<tr>
<td><strong>Plasma cell</strong></td>
<td>cytoplasmic Ig</td>
<td>PCA-1*, $T10$ (CD38)*</td>
</tr>
<tr>
<td><strong>T cell</strong></td>
<td>E-rosette</td>
<td>$T3$ (CD3), $T4$ (CD4), $T8$ (CD8)</td>
</tr>
<tr>
<td><strong>Monocyte</strong></td>
<td>phagocytosis</td>
<td>$MO1$ (CD11b), $MO2$ (CD14)</td>
</tr>
</tbody>
</table>

*KA contribution to B lineage markers*
1980s : Therapeutic Applications

1. Monoclonal Ab purging of autografts (CD 10, CD20, PCA-1)

2. T cell depletion of allografts (CD6)

3. Immunotoxins (CD19, CD38 blocked ricin)
1990s to Present: Bench to Bedside Studies

1. Importance of tumor-host interaction and microenvironment in MM pathogenesis

2. Cytokines - IL-6, IGF-1, TGF-β, TNFα, IL-11, OSM, VEGF, SDF-1α, BAFF

3. Growth, survival, drug resistance, and migration signaling cascades

4. Validation of novel single agent and combination targeted and immune therapies
Targeting Growth of MM in the BM Microenvironment

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 Ca²⁺ influx;
- ↑Bid cleavage, Fas & FasL, BH-3 only proteins: Bim, Bik, & NOXA

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

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

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

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

Sorazomib

Proteasome
- ↓ Chymotrypsin- and Caspase-like proteasome activities;
- ↑ Mono-ubiquitination;
- ↑ 26S Proteasome subunits
Lenalidomide in Myeloma

MM cells

Bone Marrow Stromal Cells

IL-6 ↑
TNFα ↑
IL-1β ↑

Bone Marrow Vessels

ICAM-1

Dendritic Cells

VEGF
bFGF

NK Cells
NK-T Cells


LeBlanc R et al. Blood 103: 1787, 2004
MAb Based Therapeutic Targeting of MM

Antibody-dependent Cellular Cytotoxicity (ADCC)

- Effector cells: NK cell, macrophage, neutrophil...

- ADCC

- Antibody-dependent Cellular Cytotoxicity

- FcR

- MM

- Lucatumumab or Dacetuzumab (CD40)
- Elotuzumab (CS1)
- Daratumumab (CD38)
- XmAb®5592 (HM1.24)
- SAR650984 (CD38)

Complement-dependent Cytotoxicity (CDC)

- CDC

- Complement-dependent Cytotoxicity

- C1q

- MM

- Daratumumab (CD38)
- SAR650984 (CD38)

Apoptosis/growth arrest via intracellular signaling pathways

- Apoptosis
- Growth arrest

- Via intracellular signaling pathways

- MM

- 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
Development of Rationally-Based Combination Therapies (HDAC and Proteasome Inhibitors)

Protein → Protein aggregates (toxic) → 26S proteasome

HDAC6

Panobinostat, Vorinostat, ACY241

HDAC6

dynein

Bortezomib, Carfilzomib, NPI0052, MLN9708, ONX 0912

Aggresome → Lysosome → Autophagy

HDAC6

Microtubule

dynein

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

19 FDA approvals and median patient survival prolonged 3-4 fold, from 3 to 8-10 years.
Bench to Bedside Translation of Novel Agents in Myeloma

Preclinical and Clinical Studies leading to FDA Approvals in MM

2006 Thalidomide
2005 Bortezomib (BTZ)
2006, 2014 Lenalidomide
2003, 2005, 2008 Bortezomib (BTZ)
2007 Doxil + BTZ
2012, 2015 Carfilzomib
2013, 2015 Pomalidomide
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
Monoclonal Antibody
Proteasome inhibitor
HDAC inhibitor
Future Therapies will Target Hallmark Vulnerabilities (Achilles Heels) in MM

Modulate Protein Homeostasis:
Target protein degradation
Trigger selective protein degradation

Immune Suppression:
Restore host anti-MM immunity

Genomic abnormalities:
Target and overcome mechanisms of genomic instability, target genomic abnormalities and their sequelae
Proteasome: Present and Future Therapies

Ubiquitin
Proteasome
Receptor
Deubiquitylating Enzymes (DUBs)
P5091 target USP-7

Potential
Therapeutic Targets
ATPases/
Cdc48

Immunoproteasome
PR-924

Poly-ubiquitinated proteins (proteasome substrates)
19S
20S
19S

Six Protease activities
β5, β5i
β1, β1i
β2, β2i

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

Degraded protein
Free Ub for re-cycling

26S PROTEASOME
Targeting Ubiquitin Receptor Rpn13

Song et al, Leukemia 2016; 30:1877-86.
Blockade of Ubiquitin Receptor Rpn13 with RA190 Inhibits Myeloma Cell Growth and Induces Polyubiquitination

Yan et al., Leukemia, 2016:1-6
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
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

Degronimids™
Target Protein Binding “Warhead”
Cereblon Linker
Cereblon E3 Ligase Complex
Ubiquitin Tag
Ubiquitin Tag
Proteasome “Destroy”
Recycle Degradation Machinery
Degrade

Ubiquitin 3 ligases: cereblon, VHL, MDM2

Substrates: EGFR, BTK, BRD4, USP7, rpn

Bradner et al, Science, 2015
Pom-beads, but not Thal-beads, pull-down TP53RK

IMiDs bind and inhibit TP53RK: a cereblon independent mechanism of MM growth inhibition

• 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 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 prolongs PFS in relapsed MM by 5 months versus len dex, leading to FDA approval

Lonial et al NEJM 2016
1980’s: Diagnostic Application: Normal versus Malignant B Cell Differentiation

T10 is CD38

Non-TALL

<table>
<thead>
<tr>
<th>B cell Progenitor</th>
<th>Pre B Cell</th>
<th>Isotype Diversity</th>
<th>Secretory B Cells</th>
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<tbody>
<tr>
<td>B4</td>
<td>10%</td>
<td>CALLA</td>
<td>IgM, IgD</td>
</tr>
<tr>
<td>B4</td>
<td>30%</td>
<td>CALLA</td>
<td>IgM, IgG</td>
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<tr>
<td>B4</td>
<td>40%</td>
<td>CALLA</td>
<td>IgM, ±G</td>
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<tr>
<td>B4</td>
<td>20%</td>
<td>CALLA</td>
<td>IgM, IgD, ±G</td>
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</table>

CLL

Diffuse Nodular PDL

<table>
<thead>
<tr>
<th>CALLA</th>
<th>Pre-B</th>
<th>Immature B</th>
<th>Virgin B</th>
<th>Lymphoblast</th>
<th>Plasmablast</th>
<th>PlasmaCell</th>
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<tbody>
<tr>
<td>B2</td>
<td>la</td>
<td>B1</td>
<td>B1</td>
<td>B2</td>
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<tr>
<td>B2</td>
<td>la</td>
<td>B1</td>
<td>B1</td>
<td>B2</td>
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<tr>
<td>B2</td>
<td>la</td>
<td>B1</td>
<td>B1</td>
<td>B2</td>
<td>B1</td>
<td>B1</td>
</tr>
</tbody>
</table>

D or N

Large Cell

Waldenstroms

Myeloma

Effects of Lenalidomide Pretreatment of PBMCs on Daratumumab-Induced ADCC Against MM1S Cells

Donor #1

Donor #2

% lysis

0 50 100 150 200

iso len 2 μM daratumumab 20 μg/ml

iso len 2 μM daratumumab 20 μg/ml

0 0.01 0.1 1 10 (μg/ml)

Daratumumab

PBMC/MM1S=10

Daratumamab Combination Therapy: PFS According to MRD Status at $10^{-5}$

- Lower risk of progression in MRD-negative patients
- PFS benefit in MRD-positive patients who received daratumumab-containing regimens versus standard of care

Avet-Loiseau al ASH 2016
ACY-241 HDAC 6 Inhibitor Enhances αCD38-Mediated ADCC in Primary MM Samples

- αCD38 antibody induces ADCC in primary MM samples
- ACY-241 treatment enhances αCD38-mediated ADCC
A BCMA Auristatin Immunotoxin Induces Strong Anti-MM Effects

**Apoptosis**

- MMAF released at lysosome to induce G2/M arrest followed by apoptosis

**ADCC**

- NK, Monocyte
- FcRIII

**ADPC**

- Macrophage
- FcRII
- Mφ engulfing MM
- MM cell lysis

**Inhibition of NFκB signaling**

- APRIL
- BAFF

**GSK2857916**

- Bone Marrow Stromal Cell
- Tai et al Blood 2014; Tai & Anderson 2015
BCMA-BiTE-based Immunotherapies

CD3, BCMA, Cytotoxic granule

T cell proliferation

BCMA-BiTE

MM cell lysis

Hipp, Tai et al Leukemia 2017, in press.
Targeting APRIL in MM

Immune Suppressive Microenvironment in MM

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

Depletion of cysteine induced immune suppression.

Tumor promotion and induction of PD-L1 expression.

Anti-PDL-1 Ab and Lenalidomide Enhance MM-Specific CD8+ CTLs in Presence of pDCs

Ray et al, Leukemia 2015; 29: 1441-4
Enhanced MM Cytotoxicity of Combination Immune Therapies

Pembrolizumab, Lenalidomide/Dex in RR MM

- Heavily pretreated RRMM (median 4 prior therapies); Acceptable safety profile
- ORR 50% and disease control (CR, PR, or SD) was 98%
- Phase 3 trials now underway

Pembroluzumab Pomalidomide/Dex in RR MM

- Heavily pretreated RRMM (median of 3 prior therapies)
- ORR 56%; sCR 8%; VGPR 13%; PR 29%
- Median DOR: 8.8 months
- Double refractory ORR: 55%

NCT02036502.
Vaccines Targeting MM Specific Peptides in Smoldering Multiple Myeloma

Goal is to prevent evolution of smoldering to active myeloma

• Cocktails of immunogenic HLA-A2-specific XBP1, CD138, CS1 peptides to induce MM-specific and HLA-restricted CTL responses

Clinical trials (LLS TAP Program):
Immune responses to vaccine in all patients including tetramer positive cells and type I cytokines

Lenalidomide with vaccine augments these immune response

Lenalidomide and PDL-1, HDAC 6i 241 with vaccine to induce memory Immune response against myeloma

Bae et al, Leukemia 2015
Vaccine Gradually Induces XBP1/CD138/CS1-Specific CTL in SMM patient

Stimulator: XBP1us / XBP1sp / CD138 / CS1 Peptides

Baseline (Wk 0)  
Post-2 Vac (Wk 4)  
Post-4 Vac (Wk 8)

Post- 6 Vac (1M FW)  
Post- 6 Vac (3M FW)

SUMMARY

Induction of XBP1/CD138/CS1 Peptides-Specific CTL by Vaccine
Autologous MM Cytotoxicity is Enhanced by ACY 241 + PD-L1 Ab

Bone marrow mononuclear cells + ACY241 (0.5 uM) + PD-L1 Ab (1 ug/ml)

Hideshima et al, 2016
ACY241 HDAC 6 Inhibitor Increases CD8+ and Memory CTL Proliferation to XBP1 Peptide Stimulation

Increased Total CD8+ CTL Proliferation Following ACY241 Treatment

- No Treatment
- 0.5 uM Treatment
- 1 uM Treatment

Increased Memory CTL (CD45RO+/CD8+) Proliferation Following ACY241 Treatment

- No ACY241 Treated
- 0.5 uM ACY241 Treated
- 1 uM ACY241 Treated
Anti-PDL-1 or LAG3 Enhances Proliferation of XBP1 Peptide-CD8+CTL Subsets: Checkpoint Inhibition on U266 Myeloma cells

CFSE low: CTL in Proliferation

<table>
<thead>
<tr>
<th></th>
<th>Total CD8+ CTL</th>
<th>CD28+ cells</th>
<th>CD38+ cells</th>
<th>Central Memory</th>
<th>Effector Memory</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Trt</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>10% Prolif Total CTL CFSE low</td>
<td>33% Prolif CD28+ CTL</td>
<td>32% Prolif CD38+ CTL</td>
<td>48% Prolif CM CTL</td>
<td>9% Prolif EM CTL</td>
</tr>
<tr>
<td>Anti-PDL1 Trt</td>
<td>22% Prolif Total CTL CFSE low</td>
<td>45% Prolif CD28+ CTL</td>
<td>43% Prolif CD38+ CTL</td>
<td>81% Prolif CM CTL</td>
<td>15% Prolif EM CTL</td>
</tr>
<tr>
<td>Anti-LAG3 Trt</td>
<td>63% Prolif Total CTL CFSE low</td>
<td>68% Prolif CD28+ CTL</td>
<td>72% Prolif CD38+ CTL</td>
<td>92% Prolif CM CTL</td>
<td>60% Prolif EM CTL</td>
</tr>
</tbody>
</table>
MM Patient #1: Response to CD19 CAR Therapy

Additional regimens including...
- carfilzomib
- pomalidomide
- vorinostat
- elotuzumab

sCR, MRD neg
Now d +307
TTP after ASCT #1 d190
Remission inversion

Garfall et al, NEJM 2015; 373: 1040-7
bb2121: Anti-BCMA Chimeric Antigen Receptor T-Cell Product Candidate\textsuperscript{1,2}

- Autologous T cells transduced with a lentiviral vector encoding a novel anti-BCMA CAR
- 4-1BB co-signaling motif selected to promote proliferation and persistence
- Construct demonstrated potent preclinical in vivo activity with low tonic signaling

bb2121 demonstrates low antigen-independent signaling

bb2121 improves survival and drives tumor clearance in MM mice\textsuperscript{2}

Summary and Future Directions

- bb2121 has demonstrated substantial anti-tumor activity in heavily pretreated patients with MM
  - Patients with stringent complete responses and elimination of minimal residual disease
  - 100% ORR (6/6) with doses above $5 \times 10^7$ CAR+ T cells

- bb2121 has been well tolerated, with mild to moderate cytokine release syndrome reported to date
  - No dose-limiting toxicities yet identified, and dose escalation continues

- Dosing escalation and expansion will continue to identify recommended phase 2 dose
Microarray Based Pathogenesis of Myeloma

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

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

**N** ➔ **MGUS** ➔ **MM** ➔ **PCL**

**CELL PROLIFERATION**
- 22 GENES
  - Transcription – RING1

**ADHESION**
- 52 GENES
  - Survival – TNFSF7

**DNA REPAIR**
- 91 GENES
  - Signalling – MD2, MACS
  - Structural – ADD1, VCL

Genomic Evolution in Myeloma and Patterns of Clonal Change

Bolli et al, Nature Comm, 2014

No Change

Linear Evolution

Differential Clonal Response

Branching Evolution

E

F

Hyper

t(11;14)

t(4;14)
WGS at Diagnosis

5286 substitutions

51 deletions and insertions

49 rearrangements

45 complex insertion
40 deletion other
36 deletion repeat
31 deletion m-homology

copy number

LOH
gain
WGS at Relapse
– Significant Increase in Complexity
Targets to Inhibit Genomic Instability

1. Homologous recombination (HR)
2. APEX nuclease activity
3. Pan nuclease activity
4. APOBEC activity

- Developed in vitro assays to measure HR, APEX, nuclease and APOBEC activity
- Ability to use this assays in HT screen

Achilles Heal: Low YAP1 Expression in Subsets of Hematological Malignancies

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

Apoptosis

Damaged DNA

ATM

Nuclear ABL1

p73

STK4

Damaged DNA

ATM

Nuclear ABL1

Ub

YAP1

Pro-apoptotic genes, cell cycle genes.

Cottini et al Nat Med 2014;20:599-606
Achilles Heal: c-MYC Amplification is Associated with Poor Prognosis

Damaged DNA

Replicative stress

MYC

Oxidative stress

ROS

PL

SOD

Apoptosis
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; 7:10258
Future Therapies will Target Hallmark Vulnerabilities (Achilles Heels) in MM

Modulate Protein Homeostasis:
Target protein degradation
Trigger selective protein degradation

Immune Suppression:
Restore host anti-MM immunity

Genomic abnormalities:
Target and overcome mechanisms of genomic instability, target genomic abnormalities and their sequelae
Conclusions and Future Directions

Combination therapies defined in preclinical studies will be used to treat subsets of patients, defined by profiling and informed by biomarkers.

Collaborative effort of academia, biotech/pharma, NIH/NCI, FDA, and advocacy- International Myeloma Society-will facilitate continued advances.

Long term disease free survival and potential cure of MM will require both 1. achieving minimal residual disease negativity, and 2. combined immune therapies to restore host immunity.
United Nations Against Myeloma: Bench to Bedside Research Team

Kenneth Anderson
Nikhil Munshi
Paul Richardson
Robert Schlossman
Irene Ghobrial
Steven Teon
Jacob Laubach
Deborah Doss
Kathleen Colson
Mary McKenney
Kim Noonan
Tina Flaherty
Kathleen Finn
Muriel Gannon
Stacey Chuma
Janet Kunsman
Diane Warren
Carolyn Revta
Andrea Freeman
Alexis Fields
Andrea Kolligian
John Feather
Farzana Masood
Nora Loughney
Heather Goddard
Tiffany Poon
Nicole Stavitzski
Ranjit Banwait
Shawna Corman
Heather Goddard
Meghan Marie Leahy
Caitlin O’Gallagher
Christina Tripsas
Karim Anderson
Shannon Viera
Katherine Redman
Amber Walsh
Samir Amin
Wanling Xie
Parantu Shah
Holly Bartel
Lisa Popitz
Jeffrey Sorrell
Teru Hideshima
Constantine Mitsiades
Dharminder Chauhan
Noopur Raje
Yu-Tzu Tai
Ruben Carrasco
James Bradner
Gullu Gorun
Jooeun Bae
Francesca Cottini
Michela Cea
Antonia Cagnetta
Teresa Calimeri
Edie Weller
Ajita Singh
Ze Tian
Diana Cirstea
Yiguo Hu
Naoya Mimura
Jiro Minami
Sun-Yung Kong
Weihua Song
Douglas McMillin
Catriona Hayes
Steffen Klippel
Jana Jakubikova
Panisinee Lawasut
Niels van de Donk
Eugen Dhimolea
Jake Delmore
Hannah Jacobs
Masood Shamas
Mariateresa Fulciniti
Jianhong Lin
Jagannath Pal
Samantha Pozzi
Loredana Santo
Claire Fabre
Anuj Mahindra
Rao Prabhala
Jake Delmore
Puru Nanjappa
Michael Sellito
Avani Vaishnav

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