Immunotherapy for Multiple Myeloma

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MIR-508
Outline

• Myeloma overview
• IMiDs
• Monoclonal antibodies
  – Anti-CS1
• Vaccines
  – Dendritic cell/myeloma fusion
Multiple Myeloma

Monoclonal protein

Malignant plasma cells

- Anemia
- Immune suppression
- Hypercalcemia

Myeloma kidney disease

Myeloma bone disease
Historical perspective of myeloma therapies

- 1840s: Rhubarb and orange peel, Steel and quinine
- 1950s: Steroids
- 1960s: Melphalan
- 1980s: Transplant
- 1990s: VAD
- 2010s: Pomalidomide (2013), Panobinostat (2015), Novel Agents
First Randomized Trial in MM

A Controlled Trial of Urethane Treatment in Multiple Myeloma


- Randomized 83 patients with treated or untreated MM to receive urethane or a placebo consisting of a cherry- and cola-flavored syrup.
- No difference was seen in objective improvement or in survival in the two treatment groups. In fact, the urethane-treated patients died earlier.

Blood 1966; 27: 328-342

Courtesy R Kyle via D Vesole
Historical perspective of myeloma therapies

1840s
- Rhubarb and orange peel
- Steel and quinine

1950s
- Steroids
- Melphalan

1960s
- Transplant

1980s
- VAD
- Pamidronate
- Thalidomide (2006)
- Zoledronic acid (2002)
- Lenalidomide (2006)
- Bortezomib (2008)

1990s
- Carfilzomib (2012)
- Pomalidomide (2013)
- Panobinostat (2015)

2000s

2010s
- Novel Agents

<1 yr
- 2-3 yrs
- >5 yrs
- >10 yrs
- Cure???
IMiDs

- Immunomodulatory effects
  - Enhancement of dendritic cell function
  - Stimulation of T cells with increased IL-2 and IFN-γ secretion
  - Enhanced NK cell number/function (upregulation of CD16 expression) → ADCC against myeloma cells
  - Inhibition of regulatory T-cells
- Cytotoxic effects
- Anti-angiogenic effects
- Disruption of the myeloma cell-bone marrow stromal cell interaction
- Teratogenic effects
Malformations due to maternal ingestion of thalidomide (Schardein 1982 and Moore 1993).
- Cereblon forms an E3 ubiquitin ligase complex
- This complex tags proteins with ubiquitin, marking them for proteolysis
- IMiDs bind to cereblon, activate the E3 ligase activity
- Zn-finger-containing transcription factors Ikaros (IKZF1) and Aiolos (IKZF3) are bound by cereblon and are transcriptional regulators of B and T cell development
Ikaros and Ailos

- Multiple isoforms due to alternative splicing, some behave as dominant negative isoforms upon heterodimerization
- Can act as both repressor and activator of gene transcription
- Essential for development of lymphoid cells
- Overexpression of dominant negative isoforms associated with some hematological malignancies, but not myeloma
CS1

- CS1 is a member of the signaling lymphocyte activating-molecule family (SLAM) of cell surface receptors (aka SLAMF7, CRACC, CD319), a subset of the immunoglobulin superfamily of receptors.
- Extracellular domain composed of one variable-type Ig-like domain and one constant type Ig-like domain.
- Most SLAM family receptor are self-ligands that interact with ligands through the V-type Ig-like domain.
- CS1 has some distinctive features: different expression pattern; only one ITSM domain; two isoforms with different signaling capacities; can bind EAT-2 but not SAP.
- CS1 highly expressed in MM cells, normal plasma cells and to a lesser extent NK and CD8+ T cells.
- Function of CS1 in MM cells is unclear—may be involved in cell adhesion, cell-cycle regulation, or other growth and survival pathways.
Elotuzumab

- Humanized monoclonal anti-CS1 antibody.
- Elotuzumab binds CS1, then recruits and engages NK cells via the Fcy receptor. NK cell activation leads to degranulation and release of perforin granules, leading to MM cell death (ADCC).
- Elotuzumab may also directly activate NK cells.
- Preclinical studies demonstrated that elotuzumab induces lysis of human MM cells when incubated with PBMCs or purified NK cells.
- Elotuzumab inhibits growth of established xenografts of human MM cells in immunocompromised mice.

Phase I study elotuzumab in relapsed/refractory myeloma

- Zonder et al., Blood 2012 120:552-9
- Every 14 day dosing
- MTD not reached up to 20 mg/kg
- Treatment-related AEs: chills (32%), pyrexia, flushing, chest discomfort, fatigue, headache, sinus tachycardia, vomiting, anorexia, dyspnea, increased creatinine
- Protocol amended to include pre-med regimen
- No responses
Elotuzumab + Lenalidomide

- Phase I study in RRMM (Lonial et al., JCO 30:1953, 2012)
  - 5-20 mg/kg elotuzumab (days 1, 8, 15, 22 cycles 1-2, then days 1 and 15 thereafter); lenalidomide 25 mg (days 1-21); dexamethasone 40 mg weekly
  - Overall response rate of 82%

- Phase II study in RRMM (Richardson et al., ASH 2012)
  - 10-20 mg/kg elotuzumab (days 1, 8, 15, 22 cycles 1-2, then days 1 and 15 thereafter); lenalidomide 25 mg (days 1-21); dexamethasone 40 mg weekly
  - Phase III dose of elotuzumab 10 mg/kg determined
  - Overall response rate of 84%
  - 78% experienced at least 1 treatment-emergent grade ≥ 3 event

- Phase III studies underway
Elotuzumab enhances natural killer cell activation and myeloma cell killing through interleukin-2 and TNF-α pathways

Balaji Balasa · Rui Yun · Nicole A. Belmar · Melvin Fox · Debra T. Chao · Michael D. Robbins · Gary C. Starling · Audie G. Rice

DOI 10.1007/s00262-014-1610-3
SQ OPM2 xenografts in SCID mice

A

Tumor volume (mm$^3$)

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B

NK cells in xenograft Day 1 post-drug

C

NKP46+ cells/40X field

Elo=elotuzumab; Len=lenalidomide

Len/Elo-enhanced myeloma cell killing and NK cell activation in a PBL/myeloma cell co-culture model

- Healthy donor PBMCs $\rightarrow$ monocyte depletion $\rightarrow$ PBL (peripheral blood lymphocyte)
- PBLs co-cultured with lenti-GFP OPM2 cells 24-72 hrs

ICAM1

NK cell activation and myeloma killing are LFA-1 and CD16-dependent

Elo/Len significantly enhances cytokine production

Neutralization of TNFα, but not IFNγ, inhibits myeloma cell killing

Neutralization of TNFα, but not IFNγ, inhibits NK cell activation

TNFα directly kills myeloma cells
• PBMC enriched in CD56+ NK cells/MM co-culture
• Elo decreases IL-2 levels
• anti-IL2Rα increases soluble IL-2 levels
• Both Elo and Len increase the frequency of IL-2 secreting cells

Depletion of either CD56+ or CD3+ cells decreased IL-2 ELISPOTs

Detection of IL-2 ELISPOTs with FACS sorted CD3+CD56+ cells from Elo+Len-treated co-cultures
• Neutralization of IL-2 with α-IL-2 in Elo or Elo+Len-treated co-cultures compared with isotype control

• Addition of exogenous IL-2 to PBL/OPM2 (B) or NK/OPM2 (C) co-cultures does not affect myeloma cell killing in the absence of elotuzumab
Tumor antigens

• Examples
  – Unique to malignant cells (e.g., idiotype protein for B-cell malignancies)
  – Normally expressed during early development (e.g., cancer testis antigens)
  –Restricted to non-essential organs (e.g., prostate specific antigen)
  – Aberrantly expressed in malignancy (e.g., MUC1)

• T cells with specificity to tumor-associated antigens have been identified in the circulation and tumor bed of patients

• Tumor cells attenuate the development of an effective immune response by presenting antigen in the absence of the co-stimulatory molecules and stimulatory cytokines → tolerance

• Tumor cells create an immunosuppressive environment
  – Increased Tregs
  – Upregulation CTLA4- and PDL-1/PD-1-negative costimulatory pathways
  – Inhibition of maturation and function of APCs
Dendritic cell vaccine strategies

• Naïve DCs
  – Introduction of tumor antigens and immune adjuvants to the vaccine bed to recruit DCs
• Activated DCs
  – Generate *ex vivo* thru culture of progenitor populations with cytokines
• Individual tumor antigens
  – Peptide-based vaccines administered with immune adjuvants
  – RNA or DNA encoding an antigen
  – Viral-based vectors that express antigen in the context of co-stimulatory molecules
• Whole tumor cells as source of antigens
  – Allows for multiple antigens that can be targeted, including those that might be unique to a given patient
  – Tumor lysates, apoptotic bodies, whole cell DNA or RNA, tumor cells manipulated to express cytokines
• DC/tumor fusion cells
  – Patient-derived DCs with autologous tumor cells
  – Optimizes antigen presentation along both class I and class II pathways → balanced CD4- and CD8-mediated response
Vaccination with dendritic cell/tumor fusion cells results in cellular and humoral antitumor immune responses in patients with multiple myeloma

- *Jacalyn Rosenblatt,* 1 *Baldev Vasir,* 2 *Lynne Uhl,* 1 *Simona Blotta,* 1, 3 *Claire MacNamara,* 1 *Poorvi Somaiya,* 1 *Zekui Wu,* 2 *Robin Joyce,* 1 *James D. Levine,* 1 *Dilani Dombagoda,* 1 *Yan Emily Yuan,* 1 *Karen Francoeur,* 1 *Donna Fitzgerald,* 1 *Paul Richardson,* 2 *Edie Weller,* 2 *Kenneth Anderson,* 2 *Donald Kufe,* 2 *Nikhil Munshi,* 2 and *David Avigan* 1

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BLOOD, 13 JANUARY 2011 • VOLUME 117, NUMBER 2

- Phase I study, 17 patients
- Myeloma cells isolated from bone marrow aspirates (tumor-associated markers CD38, CD138, MUC1)
- Patient-derived DCs generated from adherent mononuclear cells isolated from leukapheresis product, incubated with GM-CSF and IL-4 and then TNFα
- DC/tumor fusions generated by mixing cells in present of polyethylene glycol, incubated in autologous plasma and GM-CSF
  - Quantified by determining percentages of cells that co-expressed DC and tumor-associated antigens
- SC injection in upper thigh at 3-week intervals for three doses
  - 1 x 10⁶, 2 x 10⁶, 4 x 10⁶ cohorts
DCs (CD86)  

MM cells (CD38)  

DC/MM fusion (CD86-blue) (CD38-red)

Rosenblatt et al., Blood 117: 393-402, 2011
DC/MM fusion cells have activity as APCs

- Cells co-cultured with healthy donor T-cells x 5 days.
- Thymidine incorporation assay to measure T-cell proliferation

Rosenblatt et al., Blood 117: 393-402, 2011
Vaccine site reaction

A

Mononuclear infiltrate

B

CD8+ T cells

CD1a+ immature DCs
Cellular immunologic response to vaccination

- Determined percentage of circulating CD4 and CD8 T cells that recognized autologous MM cells
- Isolated PBMCs, cultured with autologous MM cell lysate
- Expression of intracellular IFNγ by CD4+ and CD8+ T cells determined by intracellular FACS analysis

Rosenblatt et al., Blood 117: 393-402, 2011
Vaccine induces cancer antigen-specific response

Expansion of CD8+ T cells binding MUC1 tetramer

Rosenblatt et al., Blood 117: 393-402, 2011
11/16 evaluable patients had stable disease following vaccination

- 3 patients with ongoing stable disease (12, 25, and 41 months)
- 8 patients with stable disease 2.5-5 months post-vaccination