Molecular Targeting and HSCT

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Can GVHD/GVT be separated?

- Most likely yes but very difficult since involves same initiating cell-type and numerous variables that can affect both
- May be more a matter of “degree” of separation
Molecular targeting in HSCT

- Proteasome inhibition (bortezomib, PS341)
- HDAC inhibitors (SAHA)
- Triterpenoids (CDDO)
- NFkB block (PS1145)
Potential advantages of molecular targeting agents

• Can mediate direct anti-tumor effects
• May sensitize tumor cells to immune killing
• May also sensitize to conditioning in HSCT
• Can be immunomodulating agents which can suppress GVHD
• However, this immunosuppression can also impact GVT and immune function
Bortezomib (VELCADE)

1. A proteosome inhibitor capable of exerting direct anti-tumor effects via induction of apoptosis and/or cell cycle arrest.

2. Can synergize with other mediators (i.e. TRAIL, chemotherapy) in producing anti-tumor effects.

3. Immunomodulating effects may be due to inhibitory effects on NFκB.

4. NFκB inhibition is being assessed for the prevention of rejection in solid organ transplantation as well as GVHD.
Effects on Alloreactive T cells in vitro

Allogeneic BMT Model for Effects of GVHD/GVT

Day 0
C1498 Tumor i.v.

10 950 cGy TBI

11 BMT + SC
Bortezomib

12 Bortezomib

13 Bortezomib

Monitor for survival
Histological assessment
Effects on GVHD

Question

Can bortezomib sensitize tumor cells to NK cell killing?
Natural Killer Cells

- CD3⁻, Immunoglobulin⁻ Lymphocytes
  - Mouse: DX5⁺, NK1.1⁺
  - Human: CD56⁺ (hi and lo)

- Ability to lyse tumor cells and play a crucial role in defense against cytopathic viruses

- Secrete numerous cytokines and chemokines that induce inflammatory responses and modulate functions of monocytes and granulocytes
Short-term NK Cell Killing Is Unaffected by Bortezomib

4 Hr $^{51}$Cr Release

18 Hr $^{111}$In Release

Control

20nM Bortezomib
Purging Model

BMCs

Leukemia Cell

Bortezomib

Death Receptor

NK Cells
Schema for Purging Assay

- **BMC**
  - Cocultured with C1498
  - Injected into 950cGy-Irradiated C57BL/6 Mice

- **NK Cells +**
  - 1000 IU/mL rhIL-2
  - Plate for Tumor Outgrowth

- **20nM Bortezomib**
  - 0 hr
  - 4 hr
  - 24 hr

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Outgrowth of C1498 from Purging Assay

Day 3

- BMC: + + + + + +
- C1498: - + + + + +
- 20nM Bortezomib: - - + - +
- NK Cells: - - - + +

Day 10

- BMC: + + + + + +
- C1498: - + + + + +
- 20nM Bortezomib: - - + - +
- NK Cells: - - - + +
Bortezomib Enhances NK Cell-Mediated Purging of Leukemia
Bortezomib Enhances NK Cell Killing

- Bortezomib enhances death receptor expression on target cells
- Increased sensitivity to FasL and TRAIL mediated killing following bortezomib treatment
- Bortezomib augments NK cell-mediated purging of leukemia cells from bone marrow mixtures
- Bortezomib can also kill activated NK cells
The balancing act of proteasome inhibition on GVHD/GVT
Is timing of administration critical for bortezomib and GVHD?
Increased Morbidity in Mice Receiving Delayed Bortezomib Treatment

![Graph showing percent survival over days post BMT for different treatment groups.]

- **Bortezomib 0-2**
- **Bortezomib 5-7**
- **No Bortezomib**
Late Bortezomib Administration Increases Steady State TNFR1 Expression Levels in the Gut

Lanes 1-4: Vehicle control
Lanes 5-8: Delayed bortezomib administration

TNFR1

GAPDH

Volume Ratio (GAPDH)

P < 0.005

Vehicle control
Delayed bortezomib administration
Bortezomib treatment accelerates the appearance of Th17 cells in the small intestine during GVHD.
Delayed Bortezomib Treatment Induces Severe Gut Damage

Untreated GVHD

Late Bortezomib

Small Intestine

Colon

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Role of CD4+ versus CD8+ T cells in GVH/GVT
T cell Subsets

CD4⁺ : CD8⁺ 2:1 Ratio
Th1
Th2
Th17
Treg

Central Memory
CD62L⁺CD44⁺

naïve
CD62L⁺CD44⁺

CD8⁺

Activated
CD62L⁺CD44⁺

CD8⁺

Effector Memory
CD62L⁺CD44⁺

Th1
IL-17

Th2
IL-4, IL-10

Effector memory
CD62L⁺CD44⁺

Foxp3
CD4⁺Tregs

Central Memory
CD62L⁺CD44⁺

CD8⁺

Th1
IFNγ, IL-2

CD4⁺

CD4⁺

CD4⁺
Removal of donor CD4+ T cells decreases the risk of delayed bortezomib-related lethal toxicity.
Reduction in serum TNF\(\alpha\) but not IFN\(\gamma\) with CD4+ T cell depletion

- BALB/c → B6
- PBS
- Bortezomib

**Serum TNF-\(\alpha\) (pg/mL)**
- CD4(-) SC: p<0.01
- CD8(-) SC: n.s.
- CD4&CD8(-) SC: p<0.001

**Serum IFN-\(\gamma\) (pg/mL)**
- CD4(-) SC: n.s.
- CD8(-) SC: p<0.01
- CD4&CD8(-) SC: n.s.
The absence of donor TNFa in the CD4⁺ T-cell subset results in protection from bortezomib-induced GVHD-dependent lethal toxicity.
Reduction of Th17 cells in target organs of recipients of TNFα−/− grafts.
Synergistic Anti-Tumor Responses in A20 Lymphoma Bearing Mice

Day -6

BALB/c (H2^d) → 3x10^5 A20

750 cGy TBI
10x10^6 C57BL/6 (H2^b) BMC
4x10^6 CD4^+ depleted SC

Days 0, 5, 11, 17
7.5 µg Bortezomib

Monitor for survival

7.5 µg Bortezomib
Post-transplant bortezomib therapy with CD4⁺ T cell removal results in enhanced GVT effects in advanced tumor-bearing mice.
T cell Subsets in GVHD/GVT

Irradiation

TNF
IL 6
IL 1

TGFβ

Th 17

T reg

IL 2
INFγ

CD4 T Cell

IFNγ

TNF

GVH pathology

INFγ

IFNγ

GVT

IL 2
INFγ

CD8 T Cell
CDDO

- Triterpenoid-class of naturally occurring and synthetic compounds

- CDDO is a synthetic analog of oleanolic acid and its isomer ursolic acid

![Chemical structures of Oleanolic Acid, CDDO, and CDDO-Me](image)
CDDO

• Has potent anti-proliferative properties against a wide spectrum of tumor cell types

• Inhibits production of inflammatory cytokines.

• CDDO >10,000-fold more potent than oleanolic acid in suppressing the IFNγ-induced synthesis of NO by macrophages (Honda, J. Med Chem, 2000)

• Potent inducer of the phase 2 response
  – Protects cells against oxidative and electrophile stress.
  – Dinkova-Kostova, PNAS, 2005

Can inhibit GVHD in preclinical models – Sun et al BBMT, 2007
Molecular Targeting in HSCT

- NK/T Cell
- T reg
- T Cell
- B Cell
- HDAC inhibitor (SAHA)
- Gal Cer
- IL-2
- CD28
- CD86
- MHC
- CD40
- TCR
- CD40L
- GVT↑
- ICOS
- Th2 cytokines
- Th17 driven
- GVHD
- Cytokine Storm
- ICOS blockade
- GVT Th1 driven
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