

Molecular Targeting and HSCT

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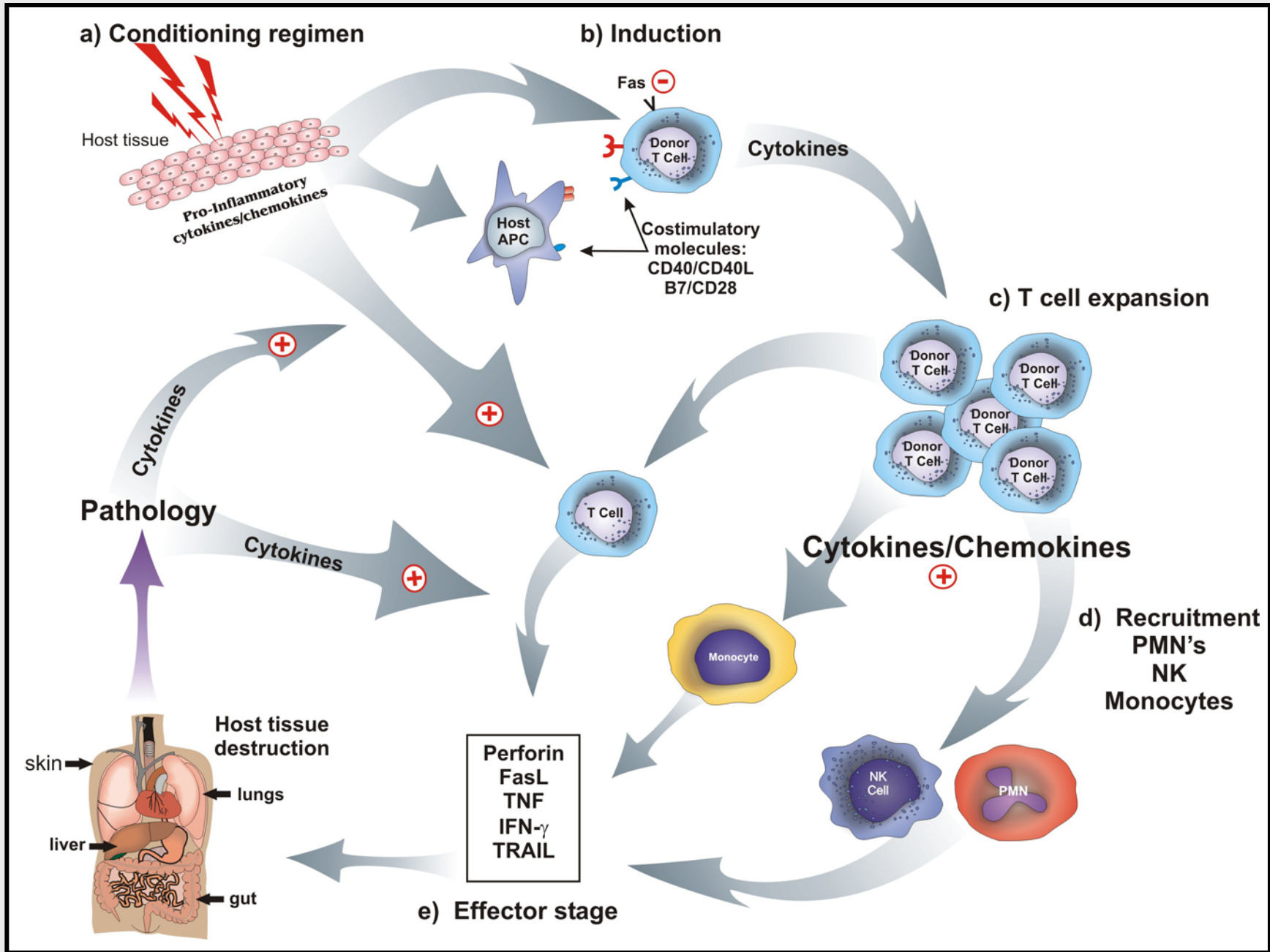
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Can GVHD/GVT be separated?

- 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



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Molecular targeting in HSCT

- Proteasome inhibition (bortezomib, PS341)
- HDAC inhibitors (SAHA)
- Triterpenoids (CDDO)
- NFkB block (PS1145)



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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



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Bortezomib (VELCADE)

- 1. A proteasome 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 NF κ B.**
- 4. NF κ B inhibition is being assessed for the prevention of rejection in solid organ transplantation as well as GVHD.**

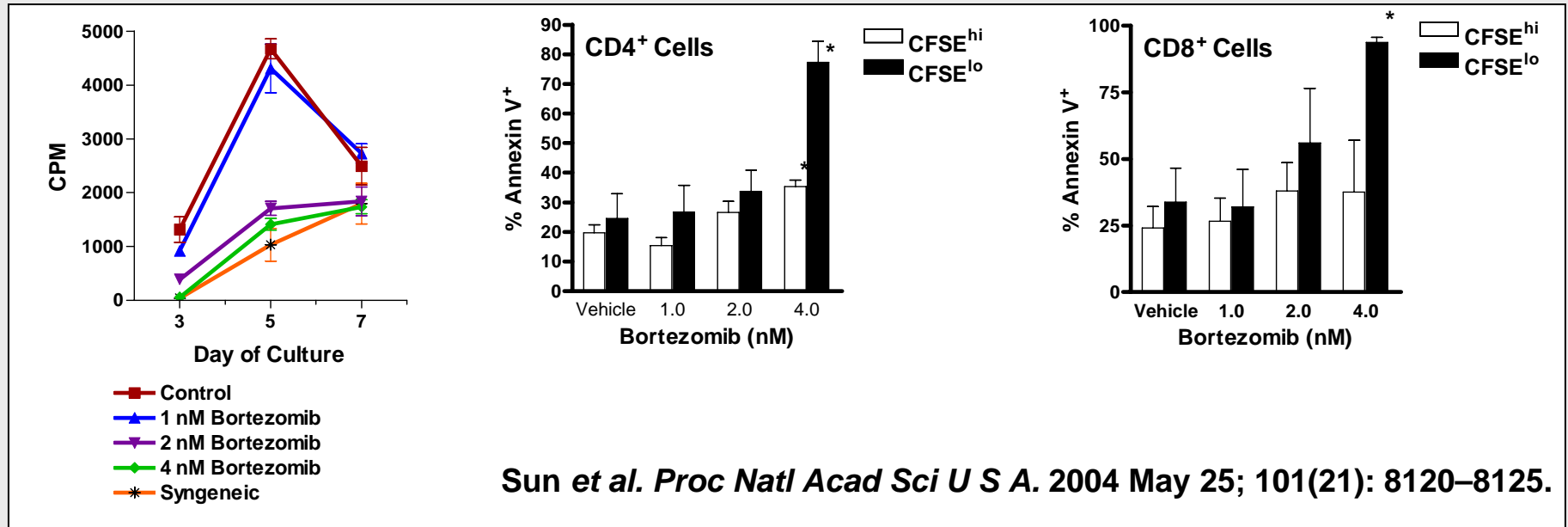


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Effects on Alloreactive T cells in vitro

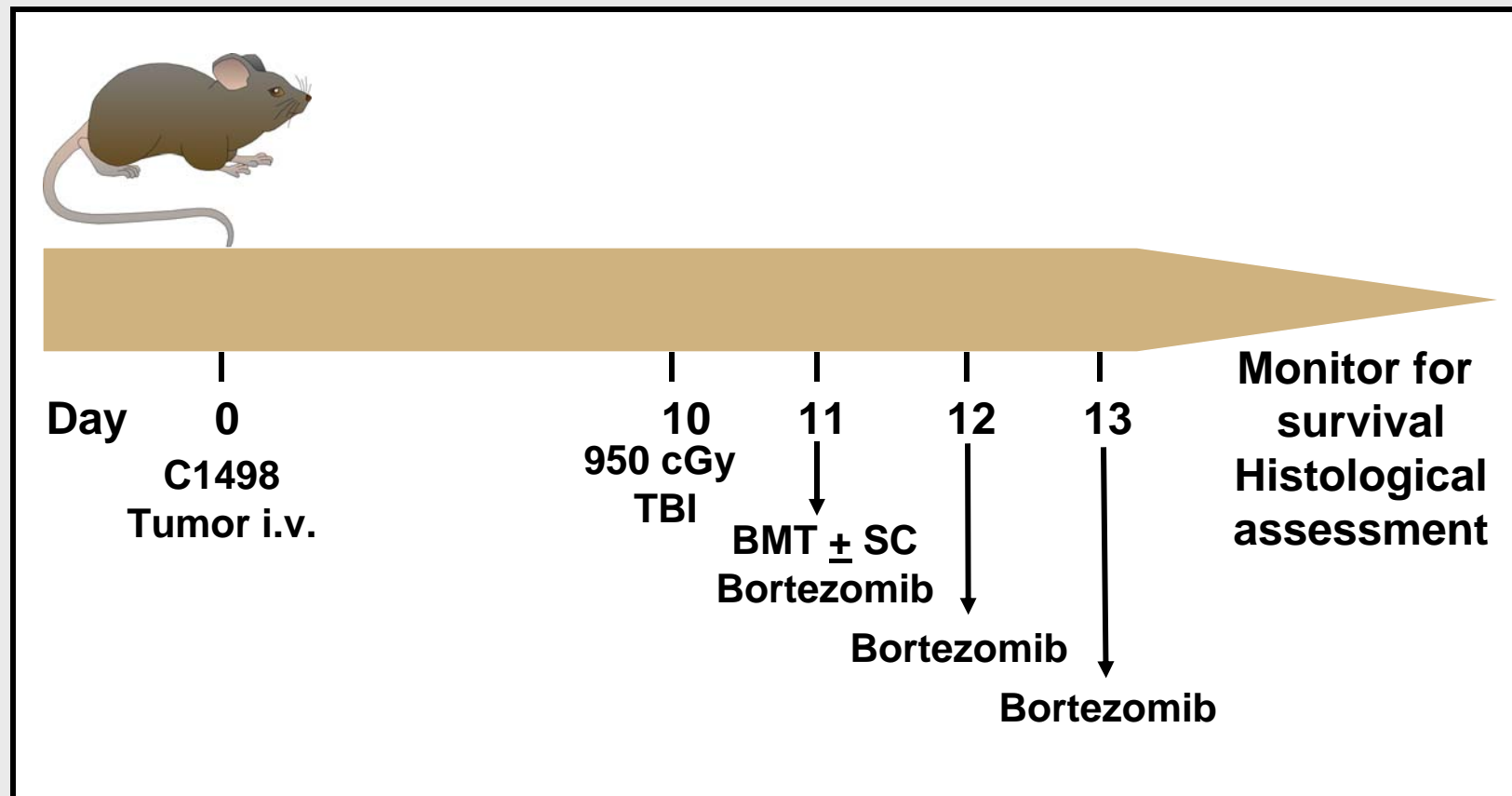


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Allogeneic BMT Model for Effects of GVHD/GVT

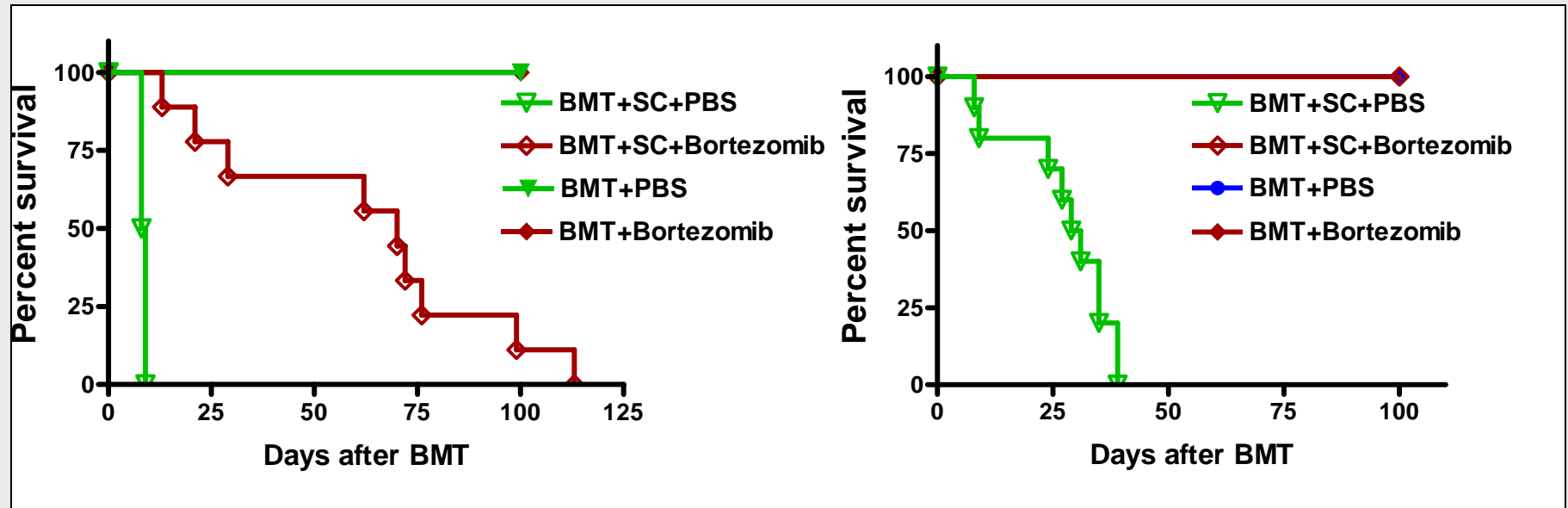


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Effects on GVHD



Sun *et al. Proc Natl Acad Sci U S A.* 2004 May 25; 101(21): 8120–8125.



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Question

Can bortezomib sensitize tumor cells to NK cell killing?

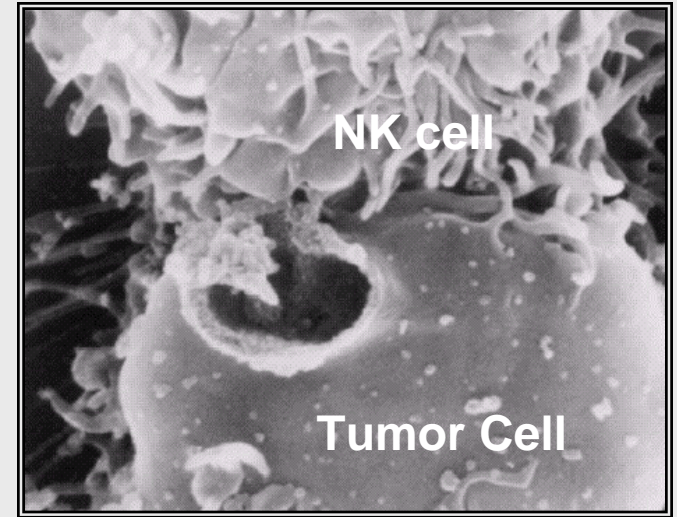


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Natural Killer Cells



- **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**

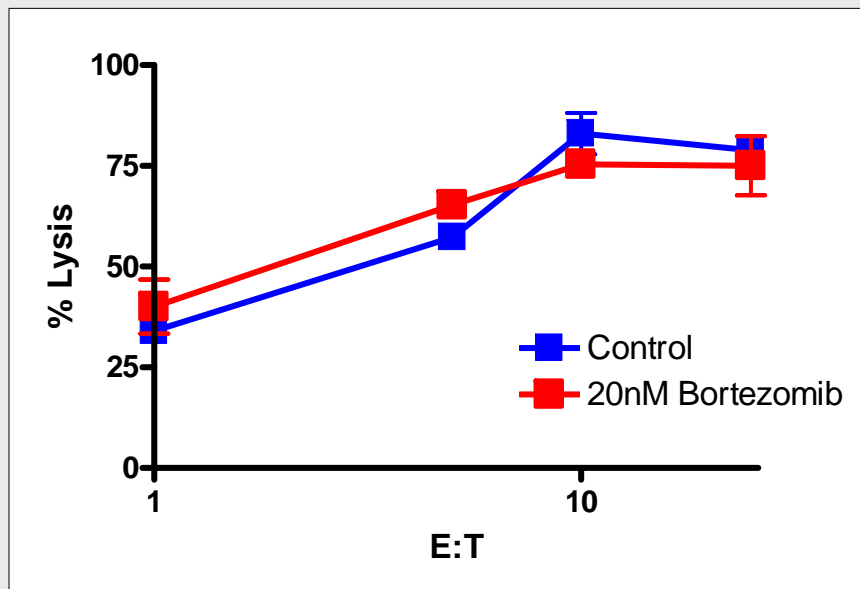


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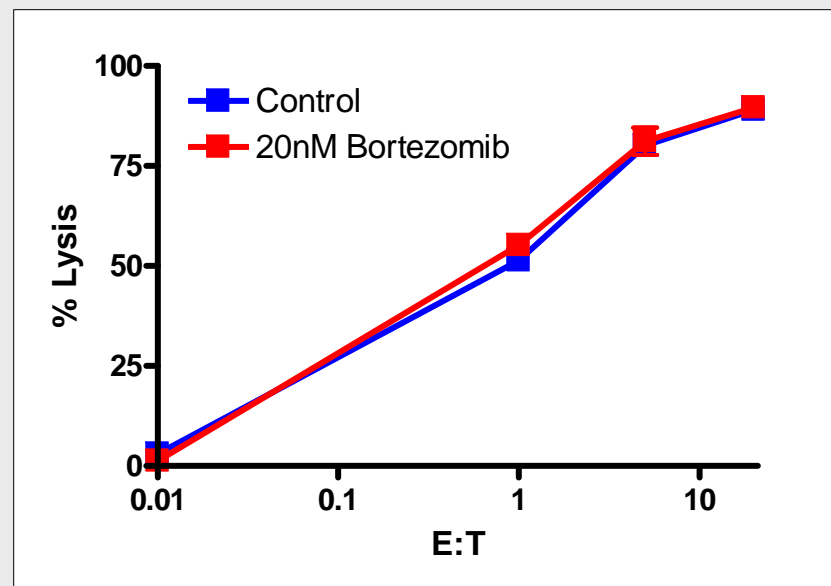
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Short-term NK Cell Killing Is Unaffected by Bortezomib



4 Hr ^{51}Cr Release

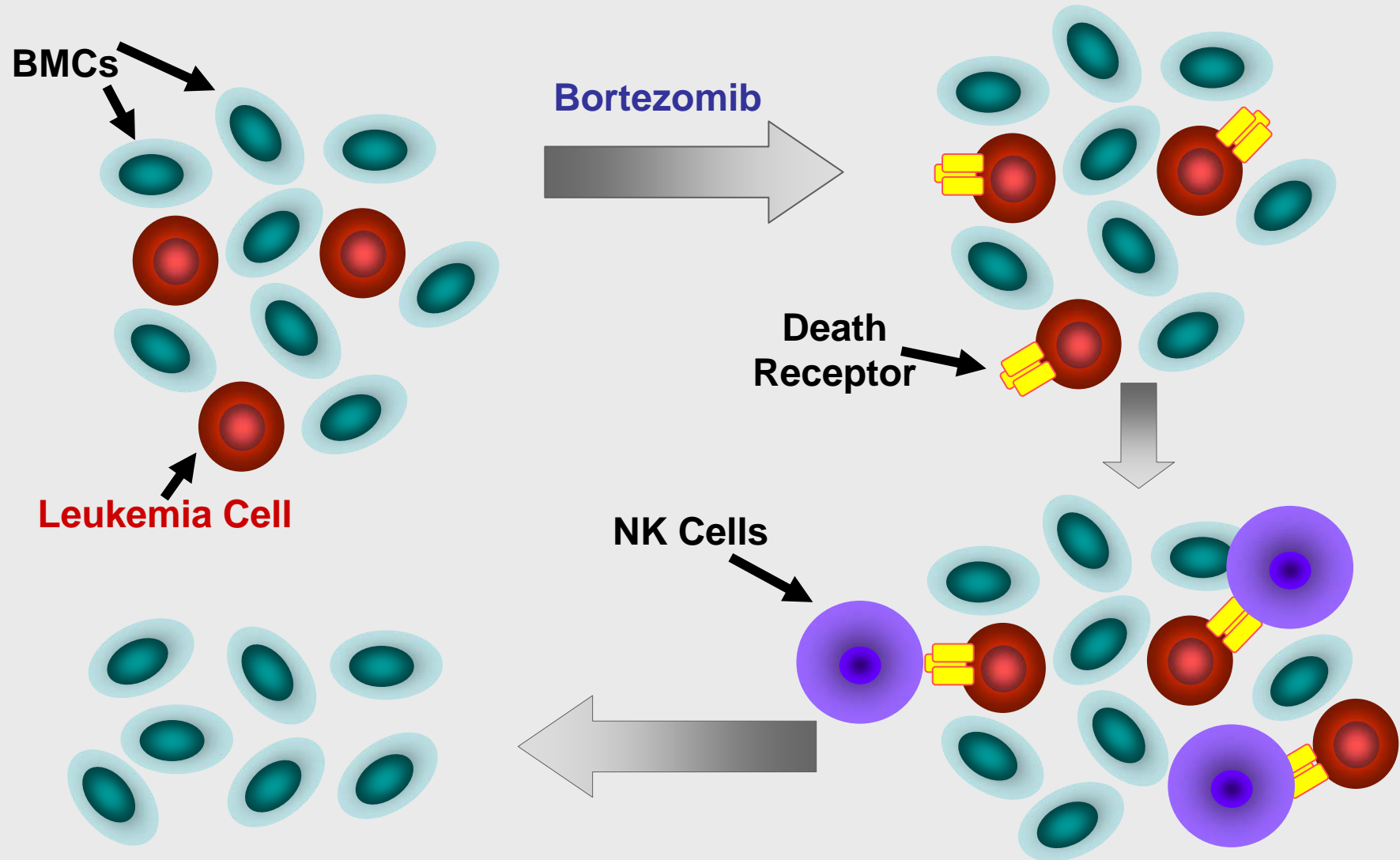


18 Hr ^{111}In Release



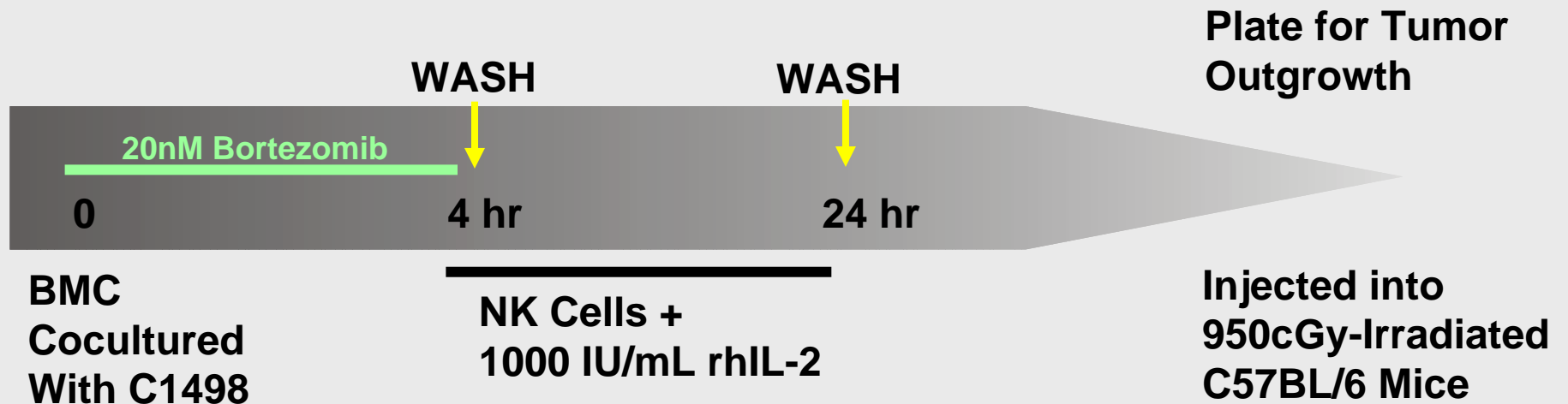
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Purging Model



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Schema for Purging Assay

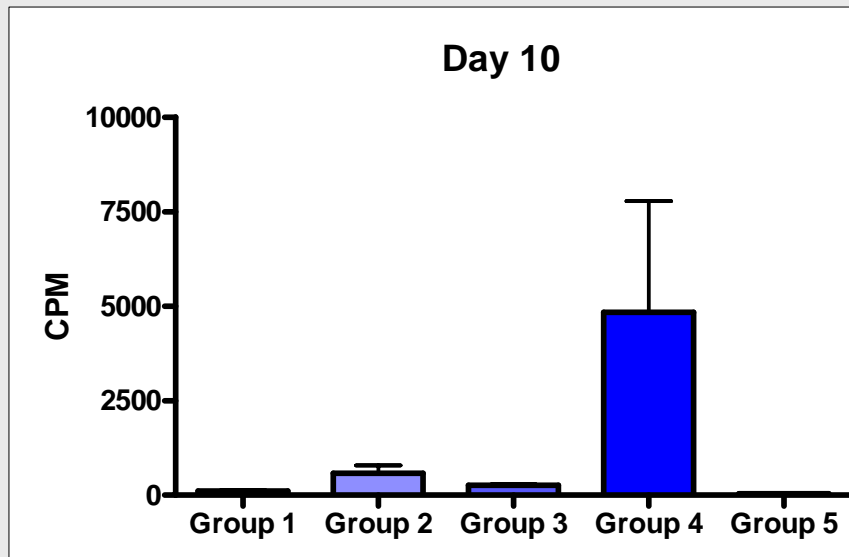
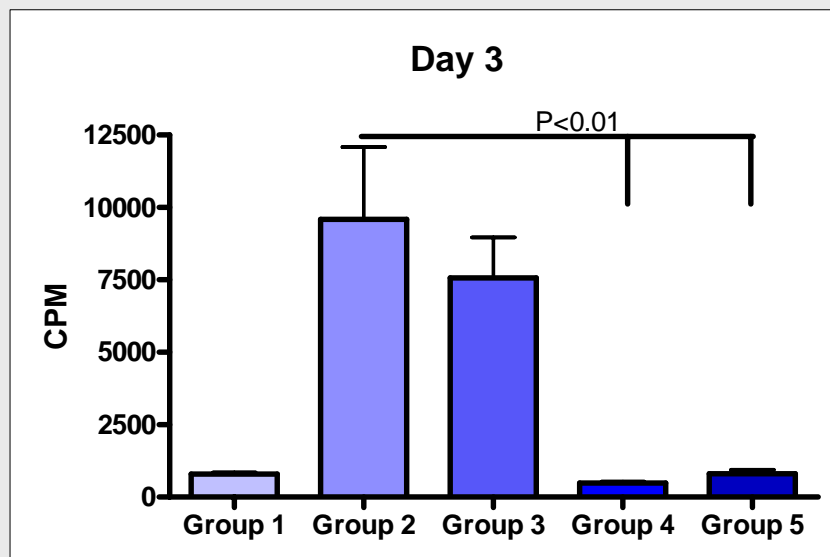


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Outgrowth of C1498 from Purging Assay



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

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

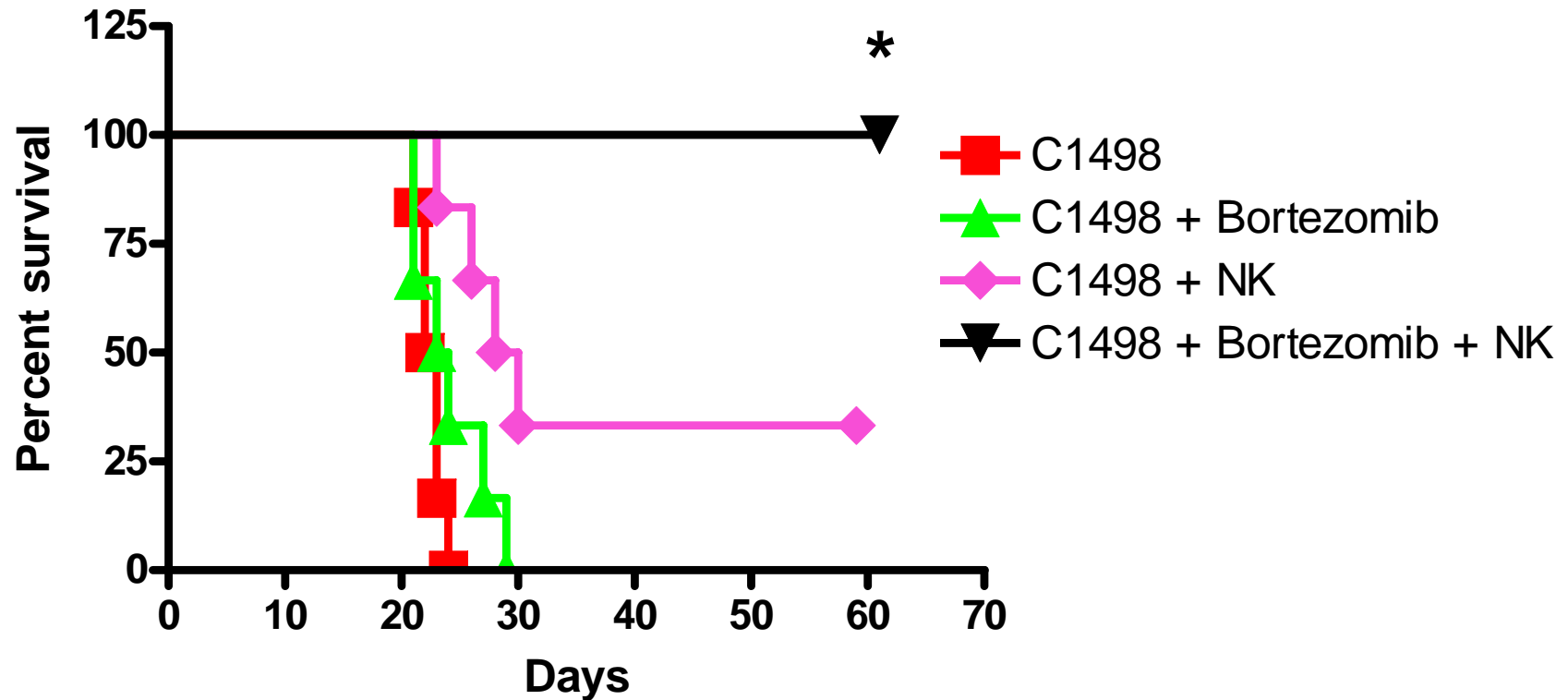


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Bortezomib Enhances NK Cell-Mediated Purging of Leukemia



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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**

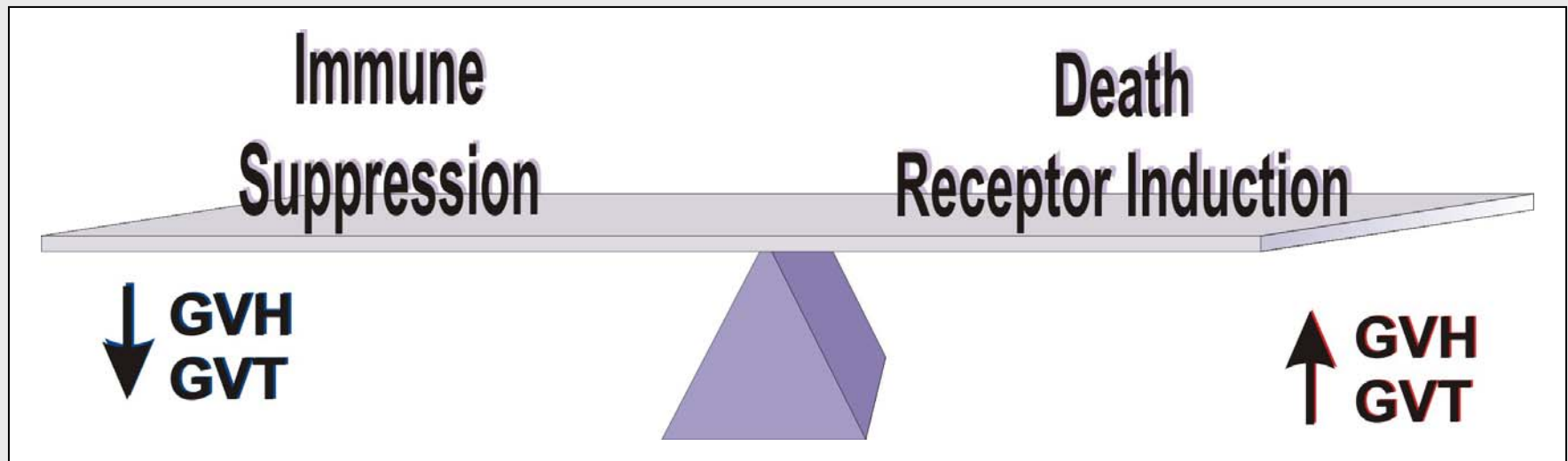


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The balancing act of proteasome inhibition on GVHD/GVT



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Question

Is timing of administration critical for bortezomib and GVHD?

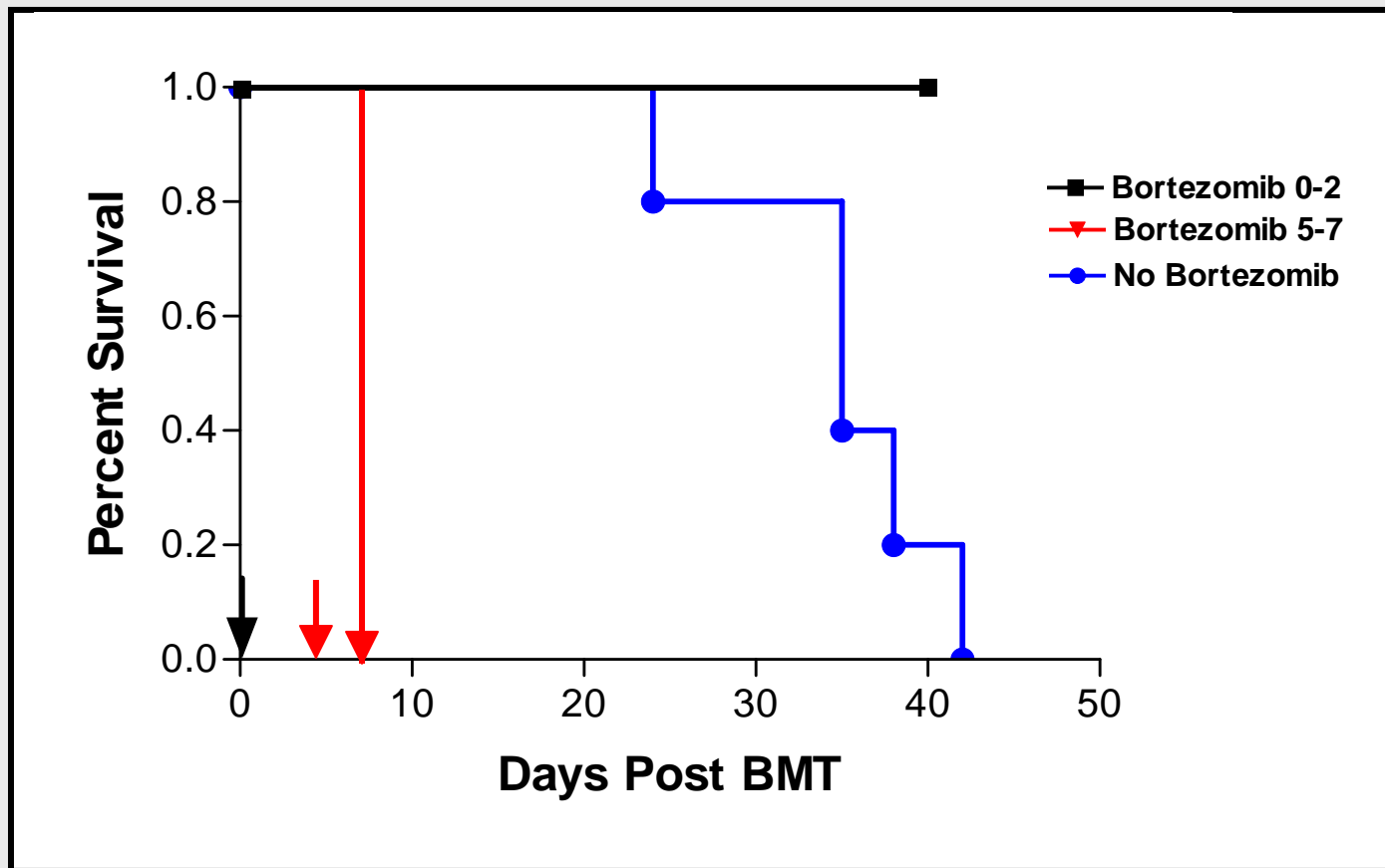


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Increased Morbidity in Mice Receiving Delayed Bortezomib Treatment

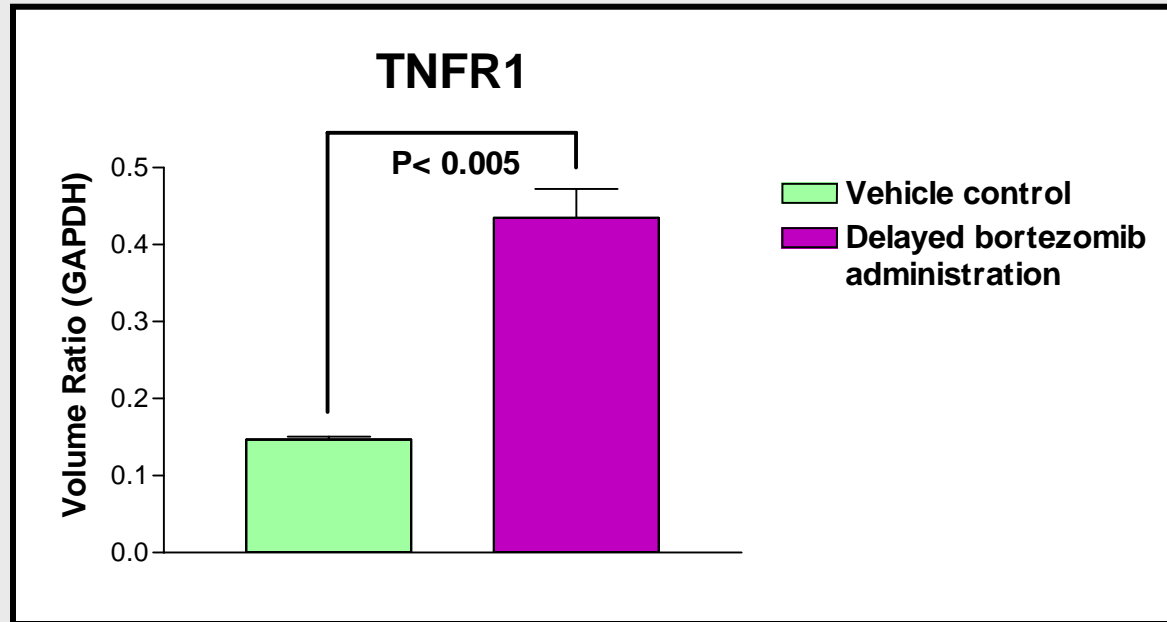
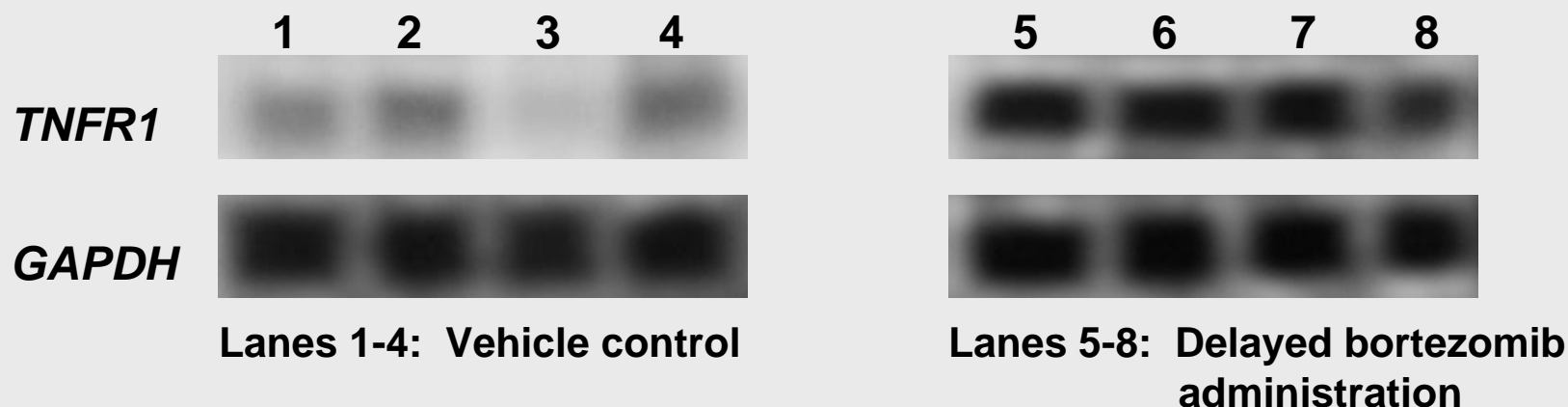


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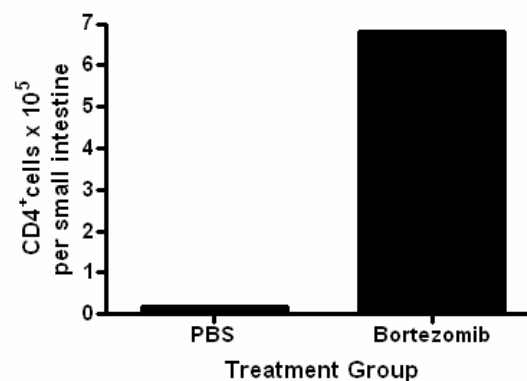
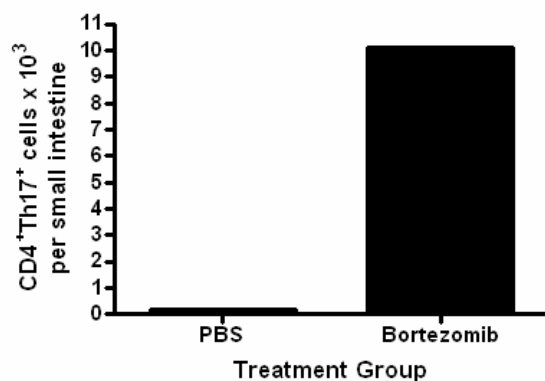
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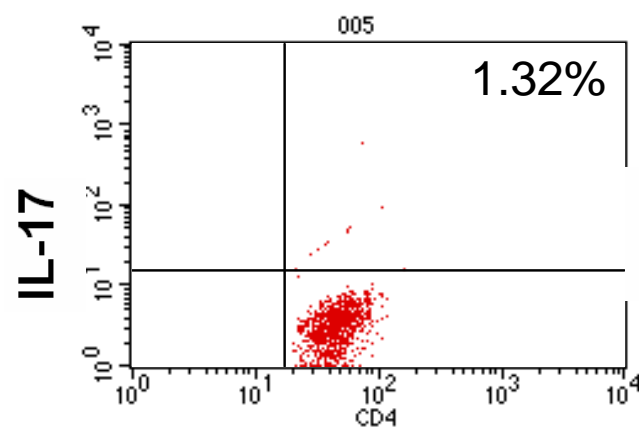
Late Bortezomib Administration Increases Steady State TNFR1 Expression Levels in the Gut



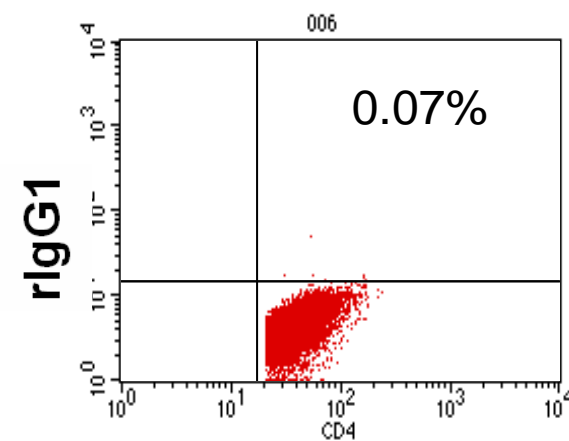
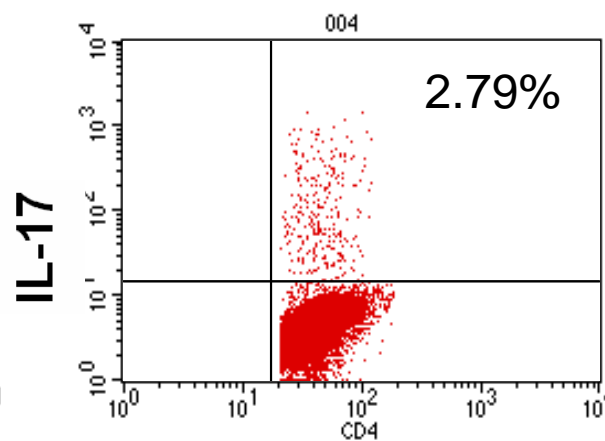
Bortezomib treatment accelerates the appearance of Th17 cells in the small intestine during GVHD



PBS treatment



Bortezomib treatment



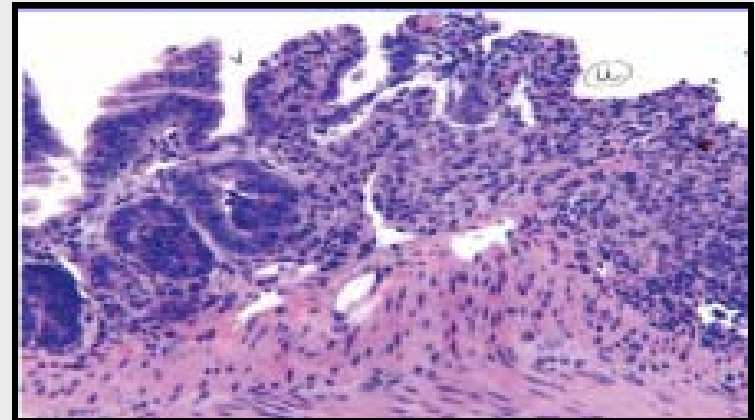
CD4

Delayed Bortezomib Treatment Induces Severe Gut Damage

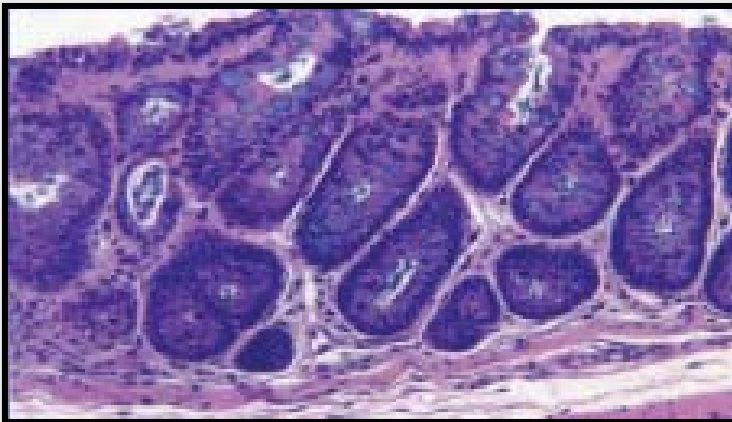
Untreated GVHD

Late Bortezomib

Small Intestine



Colon



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

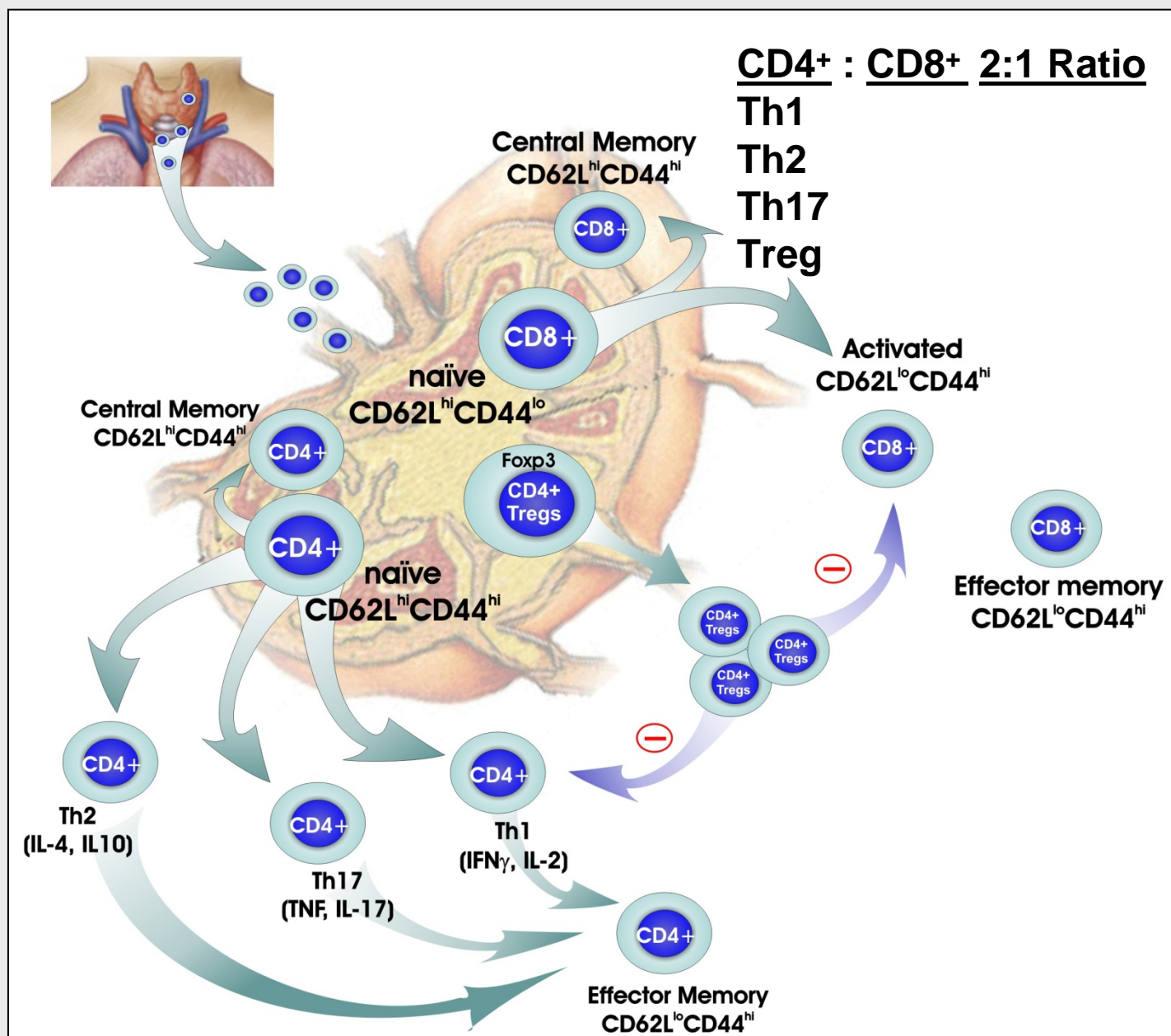


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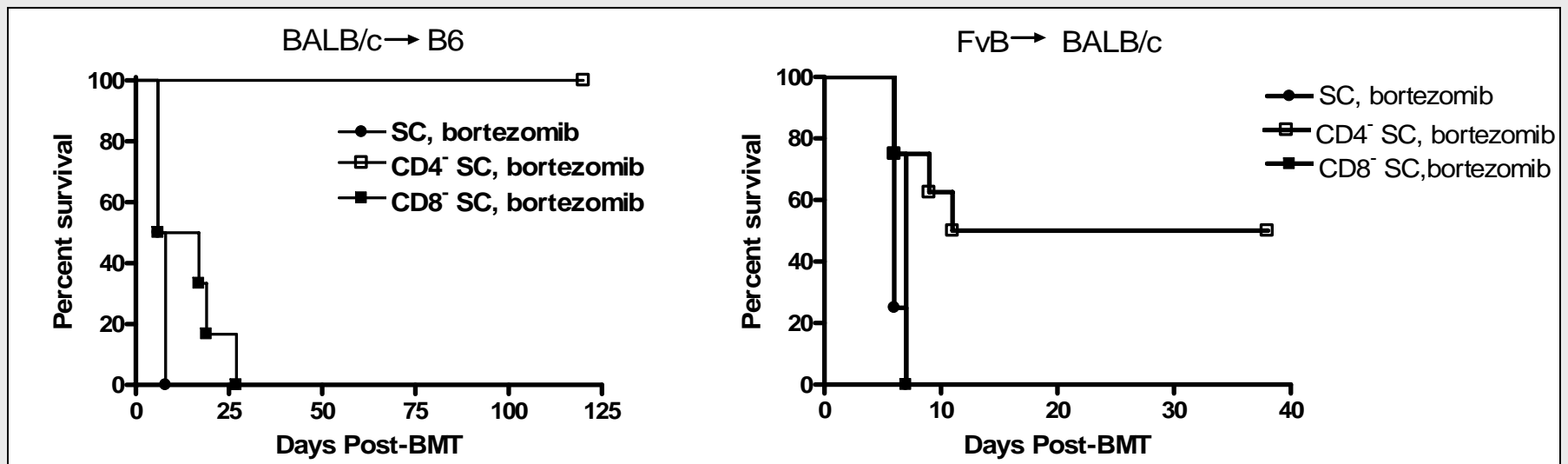
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T cell Subsets



Removal of donor CD4+ T cells decreases the risk of delayed bortezomib-related lethal toxicity.

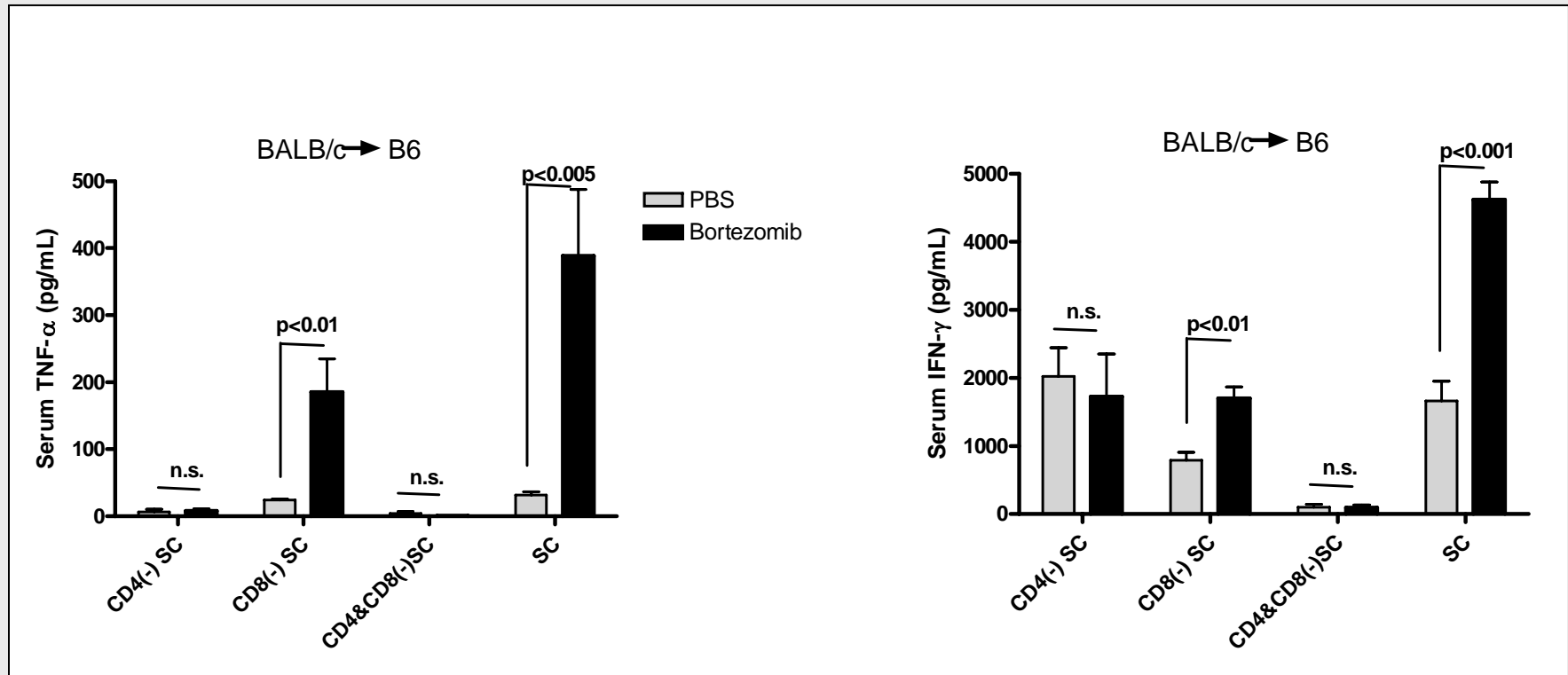


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Reduction in serum $\text{TNF}\alpha$ but not $\text{IFN}\gamma$ with CD4^+ T cell depletion

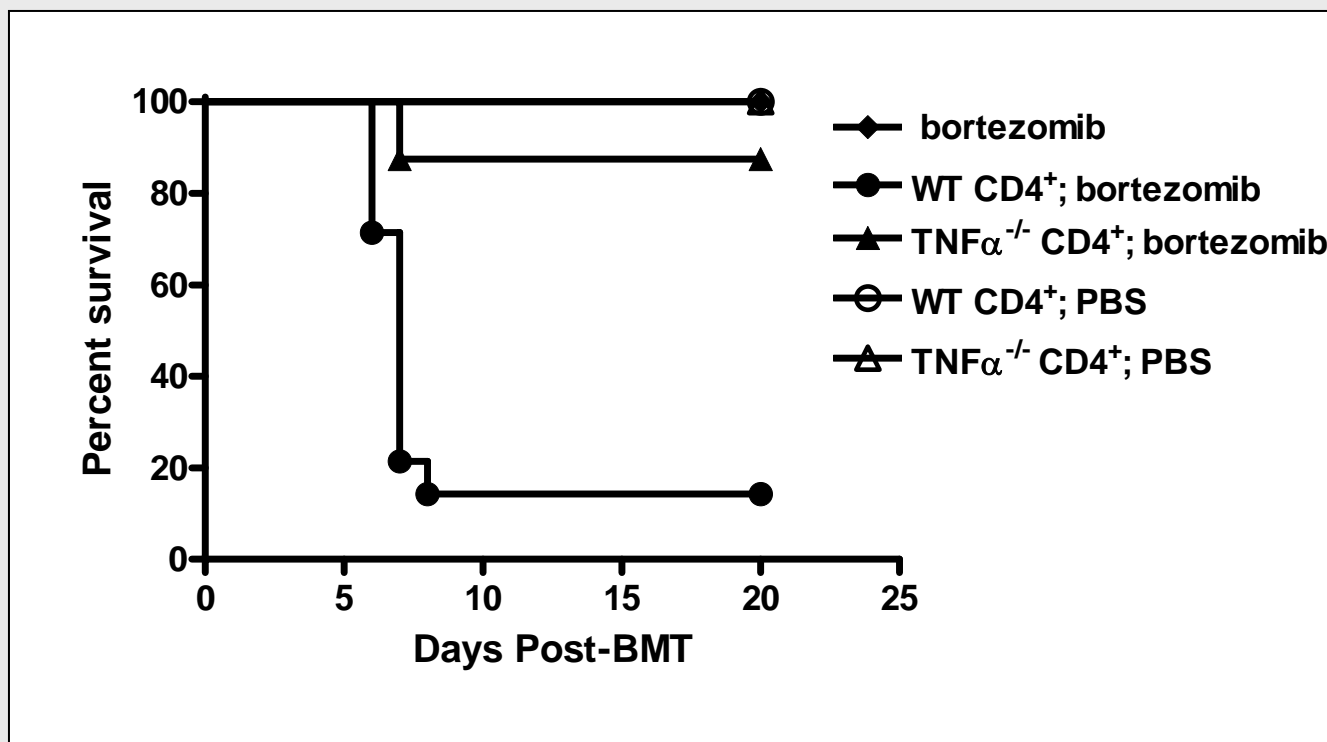


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The absence of donor TNF α in the CD4 $^{+}$ T-cell subset results in protection from bortezomib-induced GVHD-dependent lethal toxicity



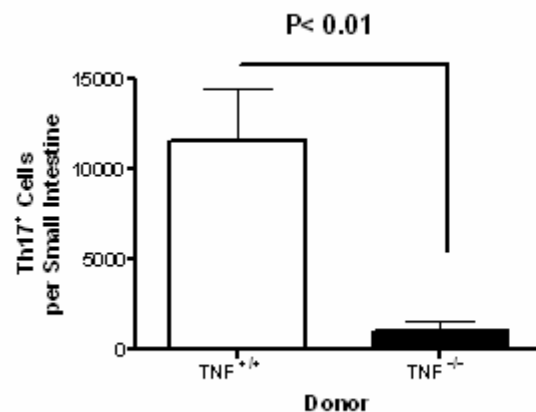
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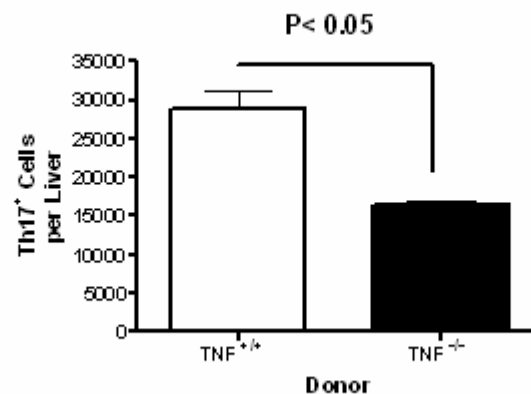
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Reduction of Th17 cells in target organs of recipients of $\text{TNF}\alpha^{-/-}$ grafts.

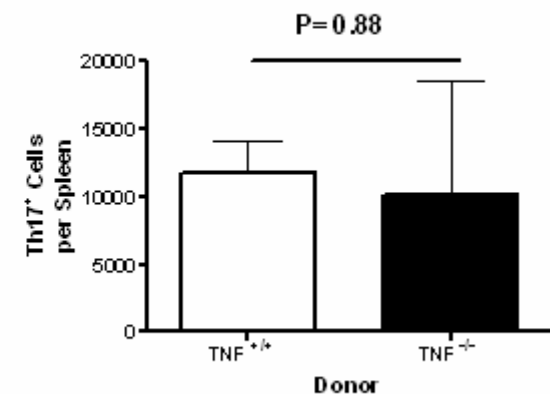
Gut



Liver



Spleen

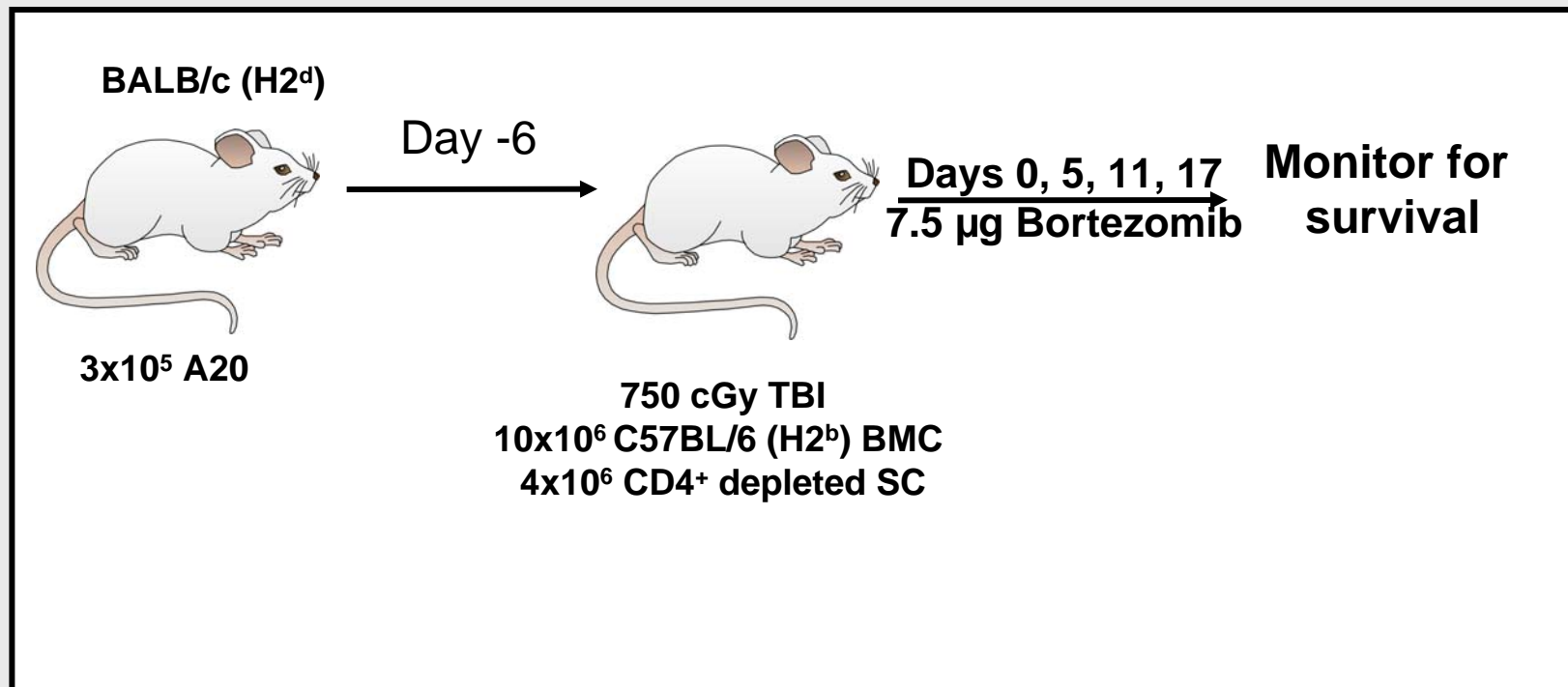


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Synergistic Anti-Tumor Responses in A20 Lymphoma Bearing Mice

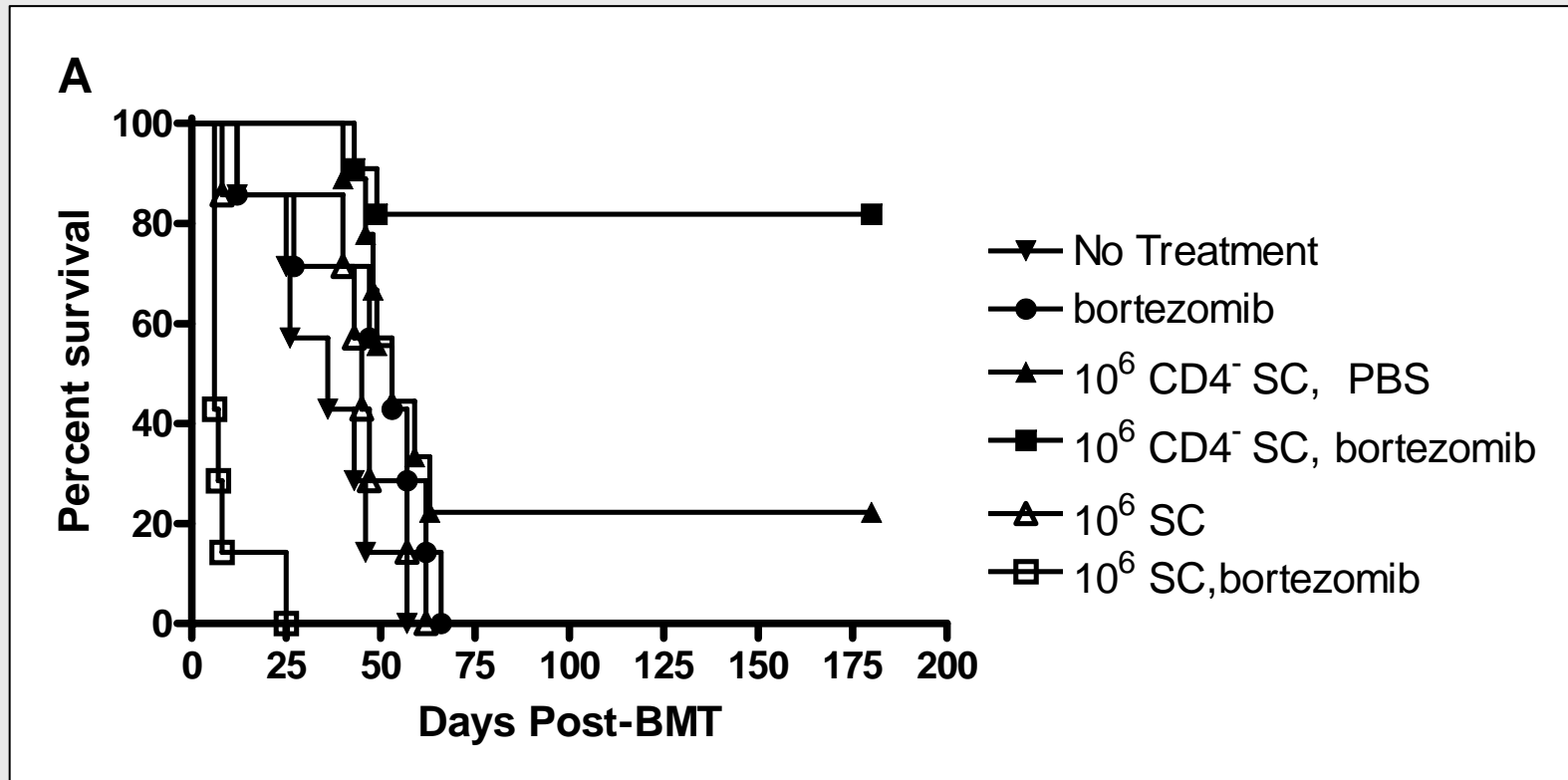


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Post-transplant bortezomib therapy with CD4⁺ T cell removal results in enhanced GVT effects in advanced tumor-bearing mice.

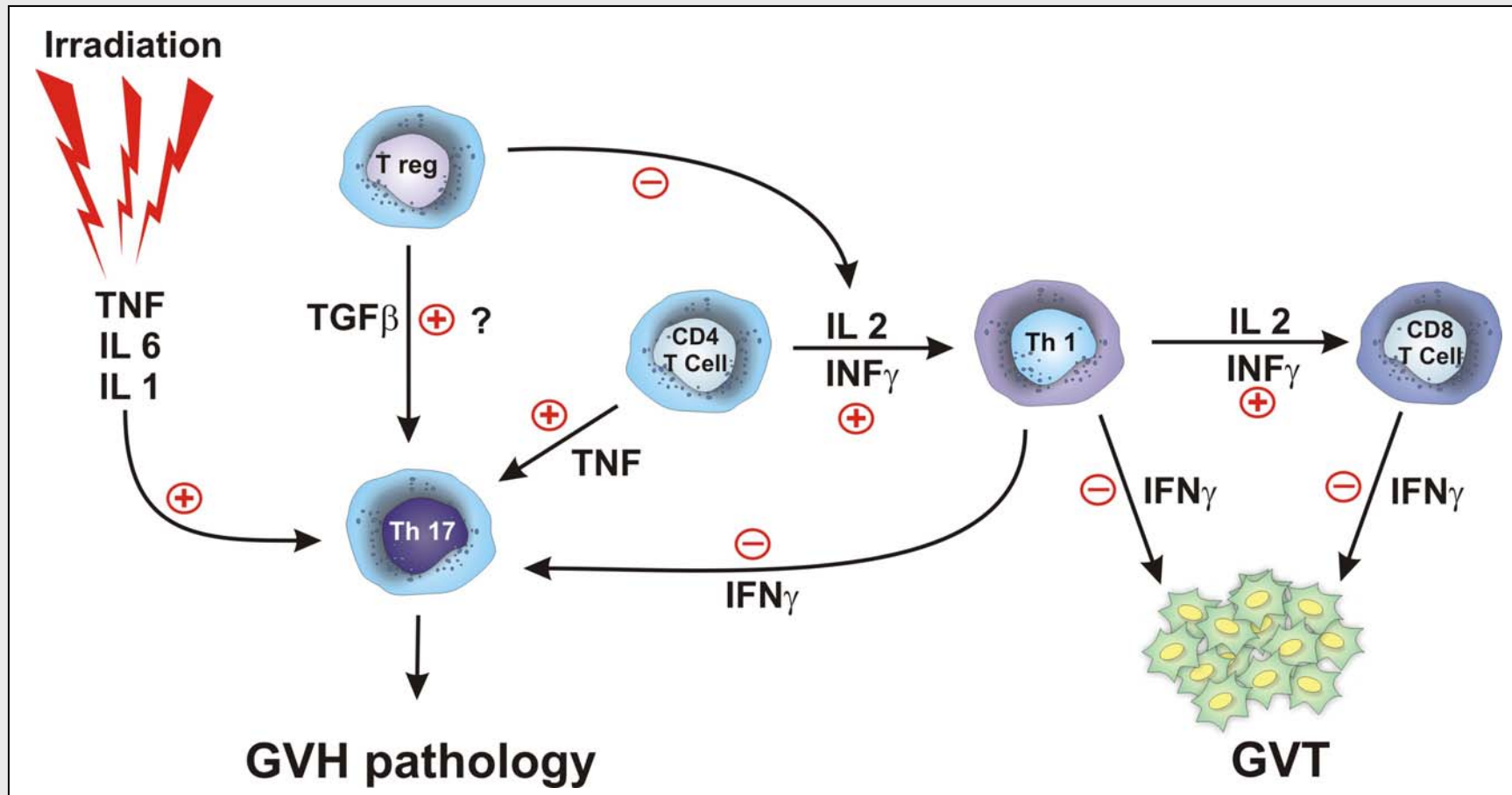


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T cell Subsets in GVHD/GVT



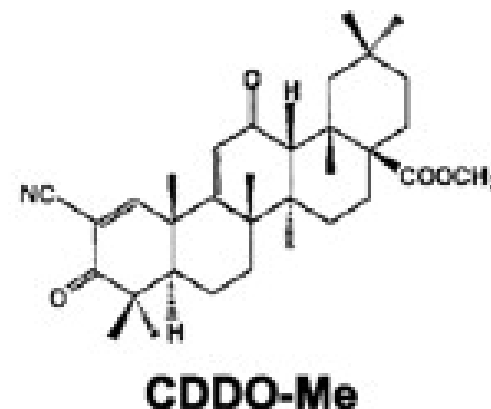
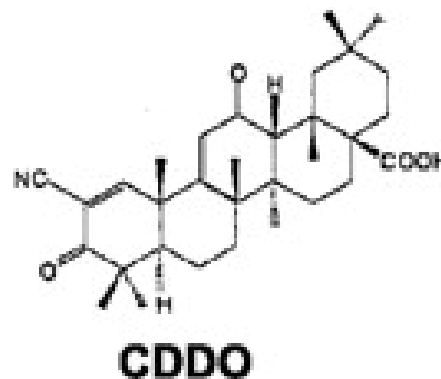
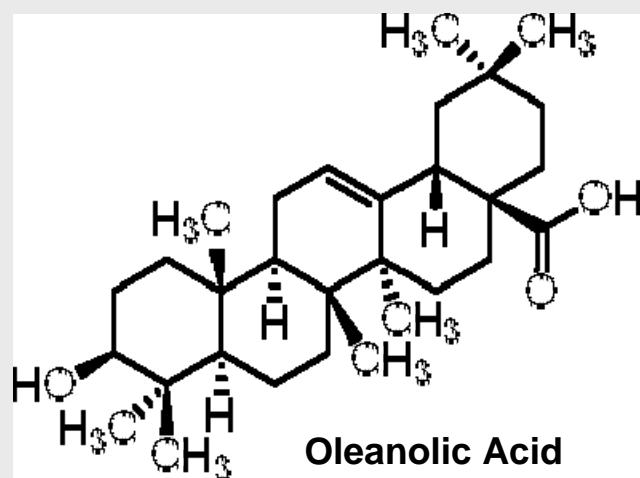
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CDDO

- Triterpenoid- class of naturally occurring and synthetic compounds
- CDDO is an synthetic analog of oleanolic acid and its isomer ursolic acid



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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 IFN γ -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.
 - Liby, *Cancer Res*, 2005.
 - Dinkova-Kostova, *PNAS*, 2005

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

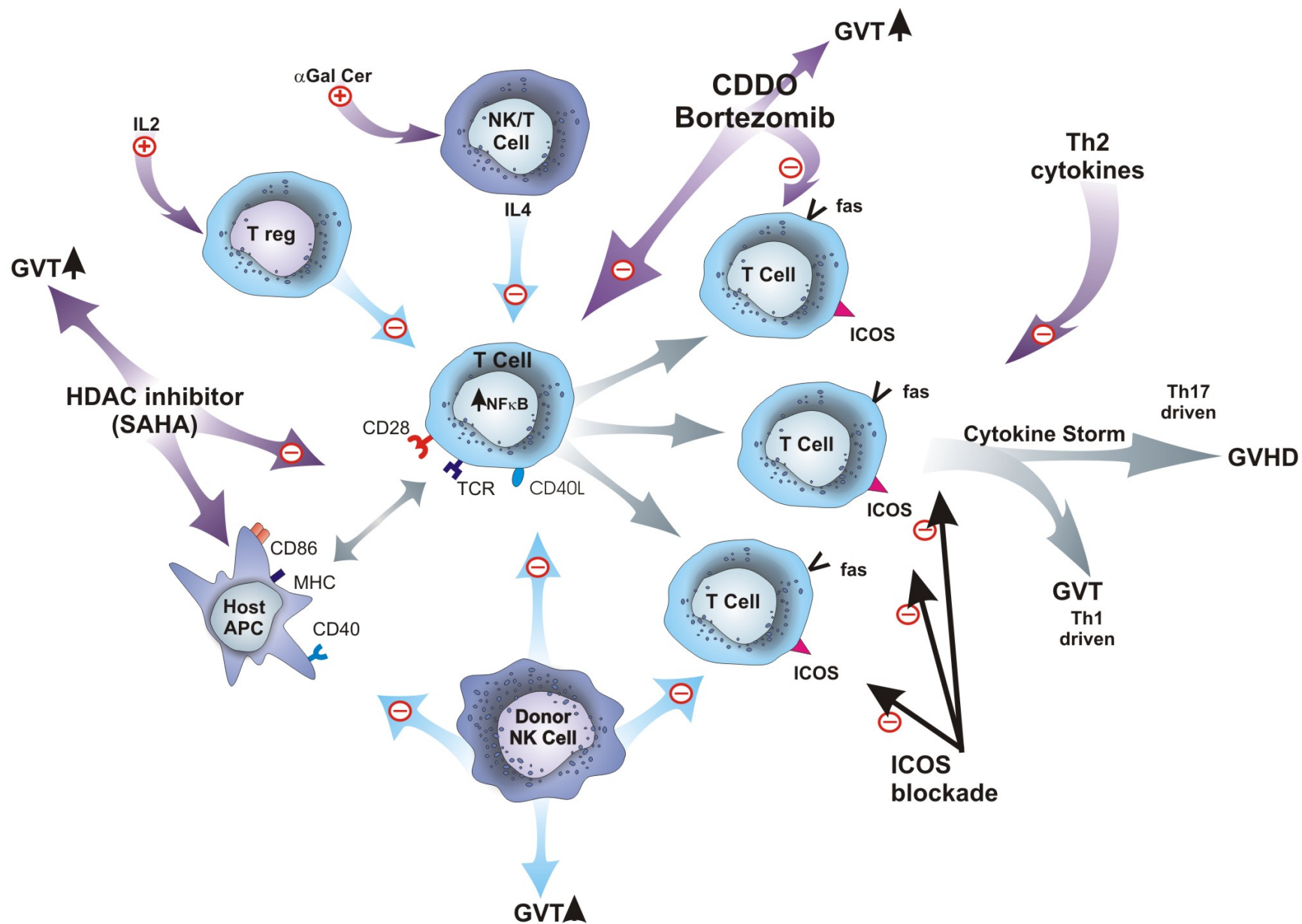


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