“PD-1/PD-L1 Blockade after Transient Lymphodepletion to Treat Myeloma”

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Myeloma

Multiple myeloma (MM) is a B cell malignancy (plasma cell origin) that primarily occurs in older individuals with a median age at diagnosis of 66 years. There are approximately 15,000 new cases per year in the U.S. and it is virtually incurable using conventional therapies (median survival in the U.S. of 2.5-3 years).

New drugs have emerged in the past 7 years including bortezomib (a proteasome inhibitor), thalidomide, and lenalidomide (a thalidomide analog), but there is no evidence that any of these agents are curative.

More recently, the treatment of choice for patients younger than 65 years of age has become high-dose therapy and autologous HSCT.

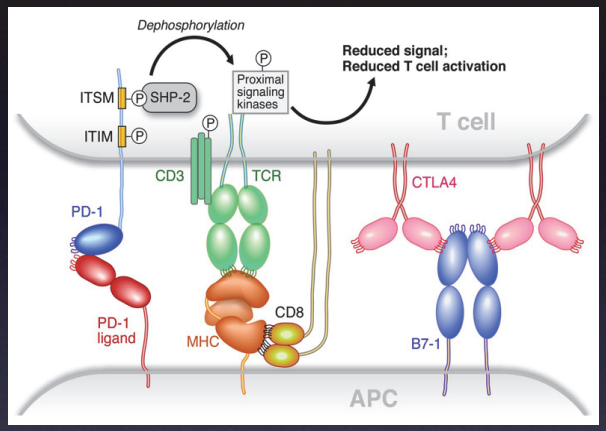
Immune-based therapies for myeloma are not being extensively studied, but they offer alternative approaches to more effectively treat these patients (particularly when combined with conventional therapies).

Overall Approach

Take clinical sample data, validate the observations in appropriate animal models, and then use the animal models to test *in vivo* relevance of the clinical observations.

Programmed Death Receptor-1 (PD-1) Pathway

PD-1 (CD279) is a CD28 family member that is expressed on the surface of activated T cells, B cells, NK cells and myeloid cells. It elicits inhibitory signals.



(Freeman GJ, PNAS, 105:10275, 2008)

On T cells, binding of PD-1 to its ligand results in tyrosine phosphorylation of the PD-1 cytoplasmic domain and recruitment of phosphatases (SHP2). Dephosphorylation of TCR proximal signaling molecules leads to attenuation of TCR signaling.

There are two known ligands for PD-1: PD-L1 (B7-H1; CD274) and PD-L2 (B7-DC, CD273). PD-L1 is expressed on many cell types including antigen-presenting cells as well as a variety of nonhematopoietic cells, and it is has been found on several different tumors. Expression of PD-L2 is more restricted and is inducibly expressed on dendritic cells and macrophages; present on some tumors.

The PD-1 pathway is involved in peripheral tolerance and appears to play a role in chronic infections and tumor immune evasion. PD-$1^{-/-}$ mice develop autoimmune diseases. PD-1 blockade can inhibit Treg function, and PD-L1/PD-1 interactions have been shown to be important in the generation of induced Treg.

PD-L1 can also inhibit T cell function by binding to CD80 (B7-1) on activated T cells.

Clinical Observations

1.Malignant plasma cells from myeloma patients express PD-L1:

-Liu et al., Blood, 110:296, 2007
-Kuranda et al., Exp. Hematol., 38:124, 2010
-Benson et al., Blood, 116:2286, 2010
-Rosenblatt et al., J. Immunother., 34:409, 2011
-Hallett et al., Biol. Blood Marrow Transplant., 17:1133, 2011

2.T cells in myeloma patients show increased PD-1 expression:

-Rosenblatt et al., J. Immunother., 34:409, 2011
-Luptakova et al., Cancer Immunol. Immunother., Epub ahead of print, 2012

Myeloma Mouse Model: 5T33

- 5T33 is a relatively non-immunogenic myeloma that spontaneously arose in a C57BL/KaLwRij mouse. It was originally maintained by in vivo passage, but eventually cultured lines capable of inducing disease were derived.
- An intravenous injection of 10^4 5T33 cells into C57BL/KaLwRij mice results in 100% lethality within 5-6 weeks. Death in the majority of animals is preceded by development of hind-leg paralysis.
- Disease is remarkably similar to human myeloma in that tumor cells primarily localize in the bone marrow, bone lesions develop, and the animals develop paraproteinemia.
- Syndecan-1 (CD138) is expressed on 5T33 cells and can be used as a tumor-specific marker. The cells also express CD45, MHC class I, CD44, CD54, low levels of CD38 and CD16/32, and Sca-1 (~20%). Expresses high levels of PD-L1, but not PD-L2.

Experimental Observations (5T33 Model)

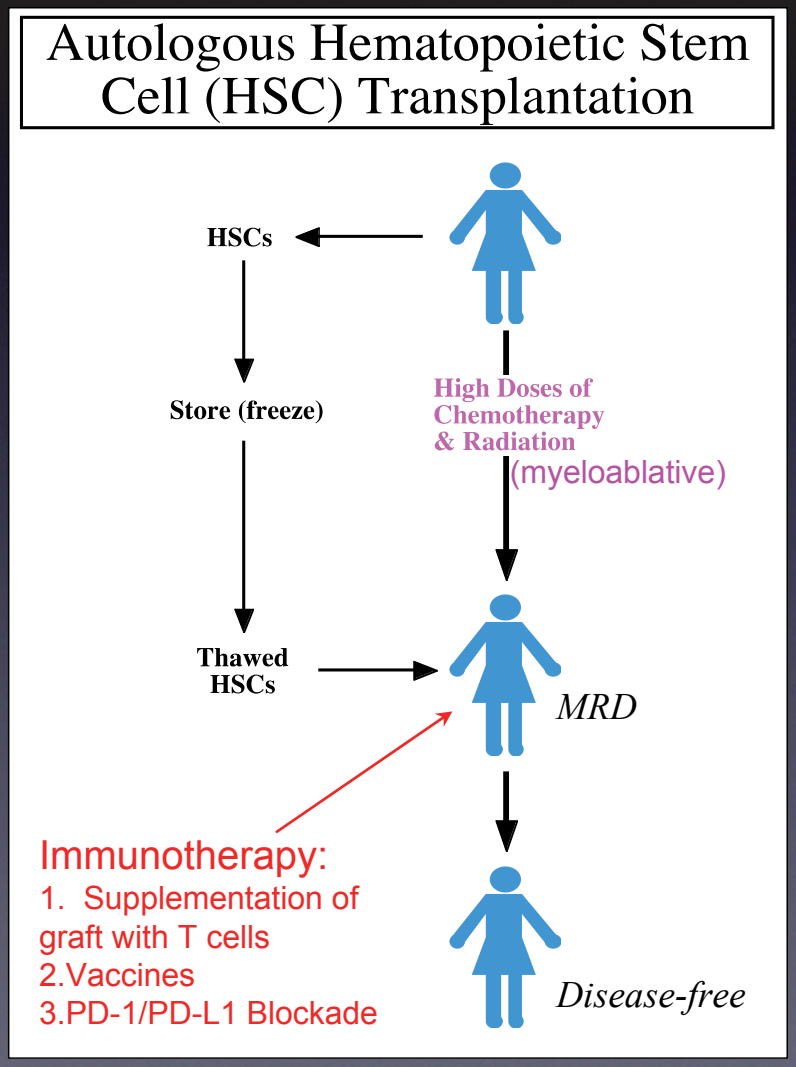
(Hallett et al., Biol Blood Marrow Transpl 17:1133, 2011)

1.Similar to the clinical data showing that malignant plasma cells from myeloma patients express PD-L1, *the mouse 5T33 cells express relatively high levels of PD-L1*.

2.Similar to the data showing that T cells in myeloma patients have increased PD-1 expression, *increased percentages of T cells in 5T33 myeloma-bearing mice express PD-1. These PD-1^{+} cells accumulate in tissues where tumor cells are present (bone marrow, spleen, liver).*

Use of Hematopoietic Stem Cell Transplantation (HSCT) as a Platform for Immunotherapy

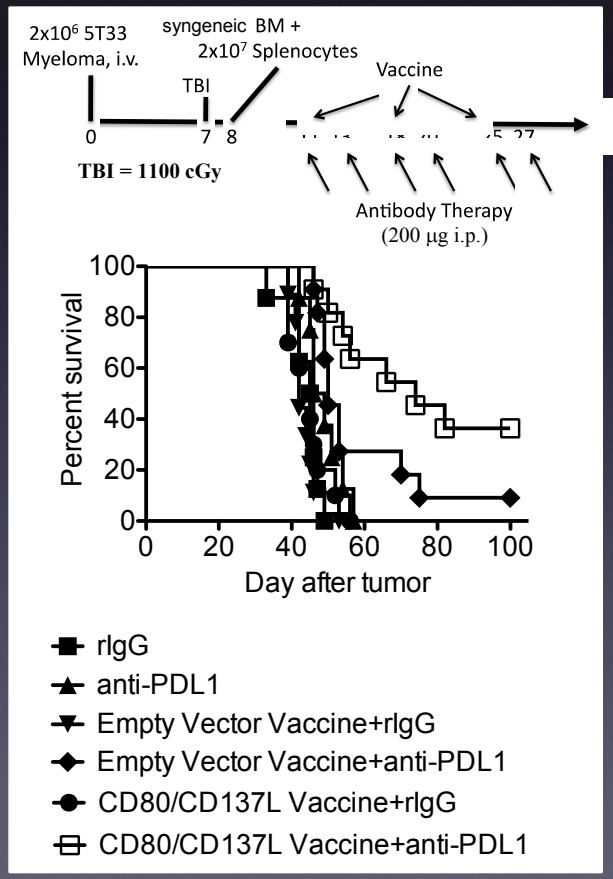
Relevance: HSCT is being used in the treatment of myeloma.



PD-L1 Blockade Improves the Efficacy of Post-HSCT Immunotherapy (T cell transfer & vaccination) for Established Myeloma

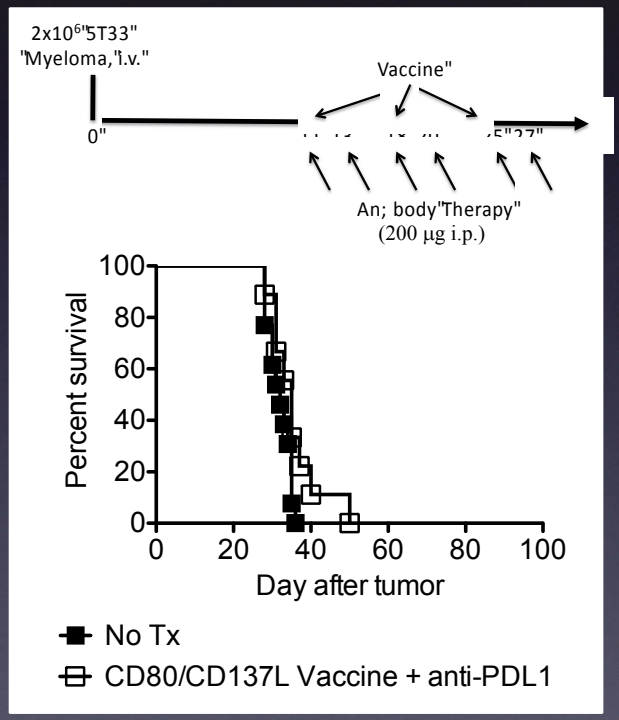
(Biol Blood Marrow Transpl, 17:1133, 2011)

Lethal TBI – HSCT



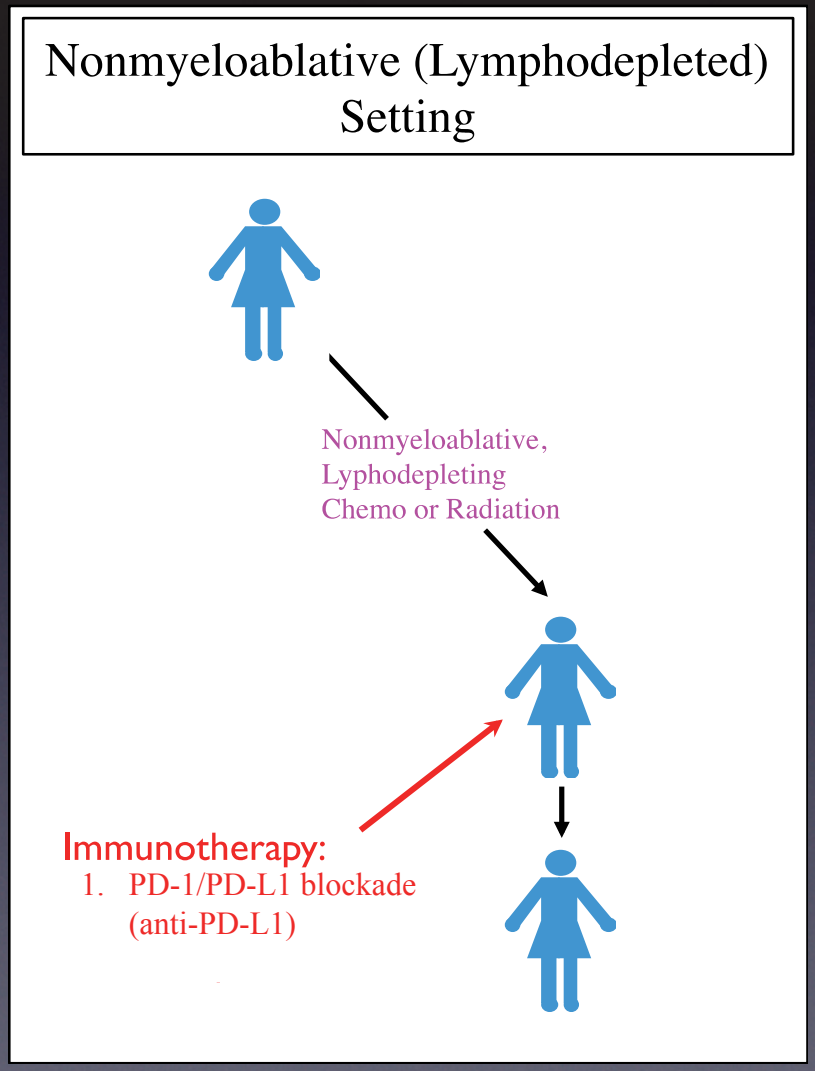
Syngeneic = Autologous

Non-Transplant

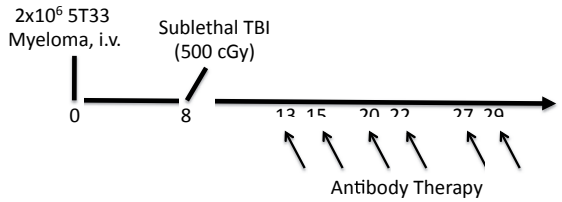


-note that 1100 cGy only extends survival by ~1 week

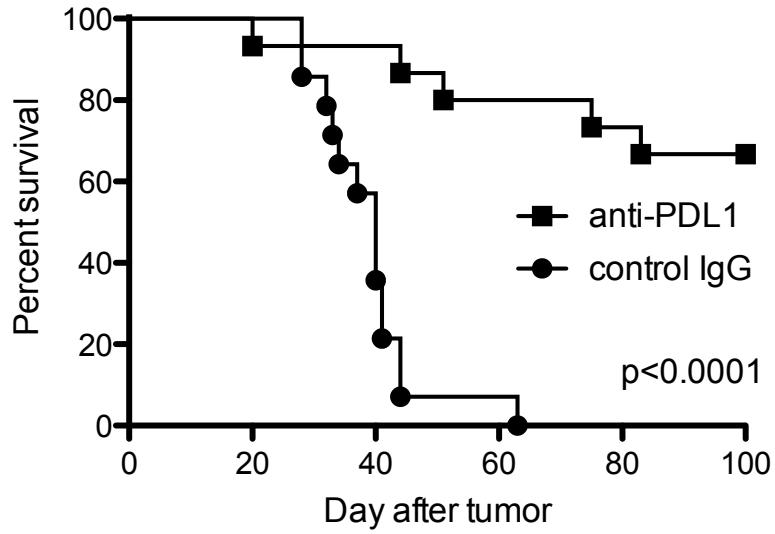
Could Rejection of Established Myeloma be Accomplished With a Combination of Lymphodepletion and PD-L1/PD-1 Blockade?



A Combination of Sublethal Irradiation and PD-1/PD-L1 Can Effectively Treat Myeloma



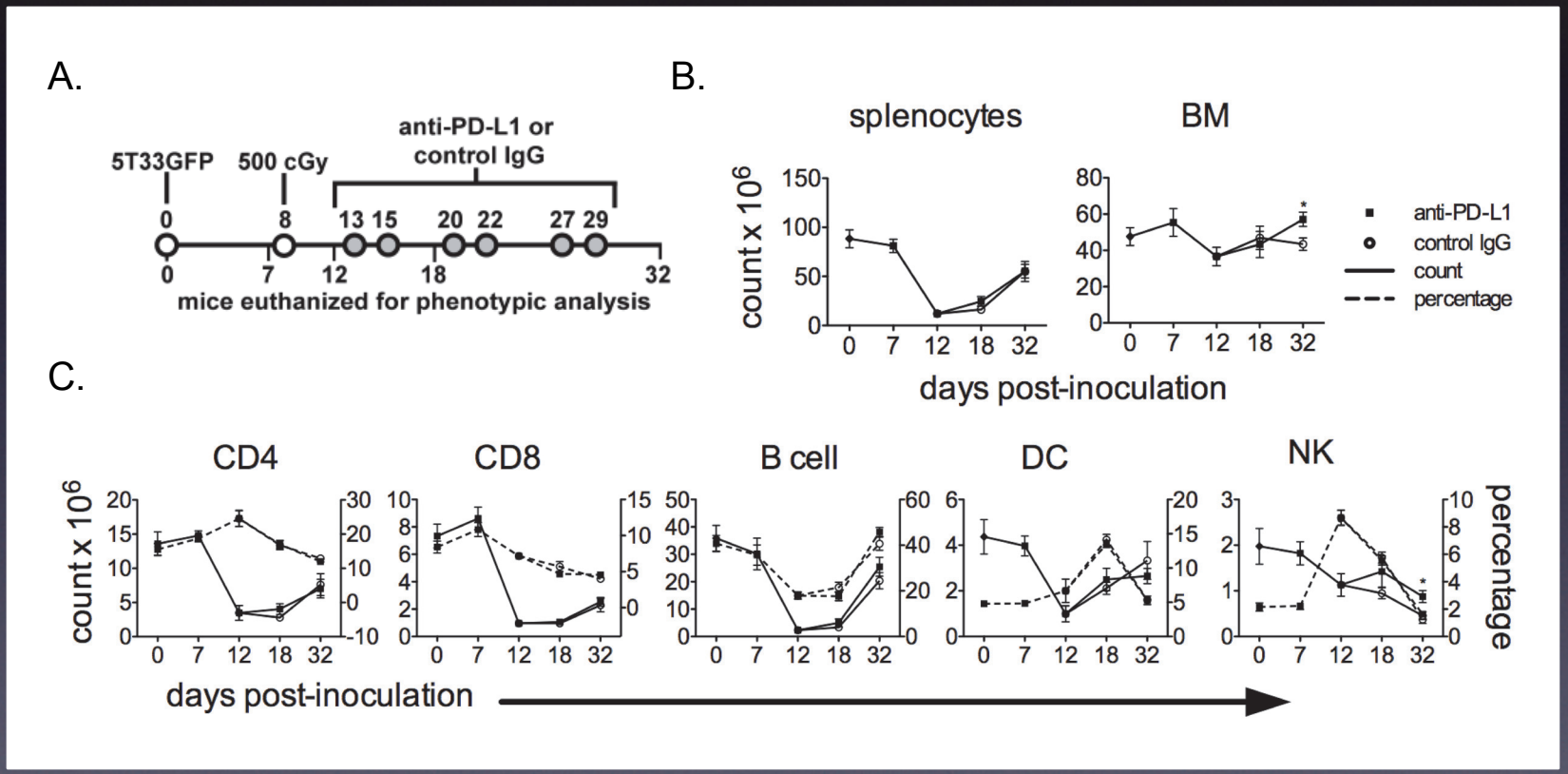
-no HSCT
-no tumor vaccines



Anti-PD-L1 without sublethal TBI is ineffective (not shown).

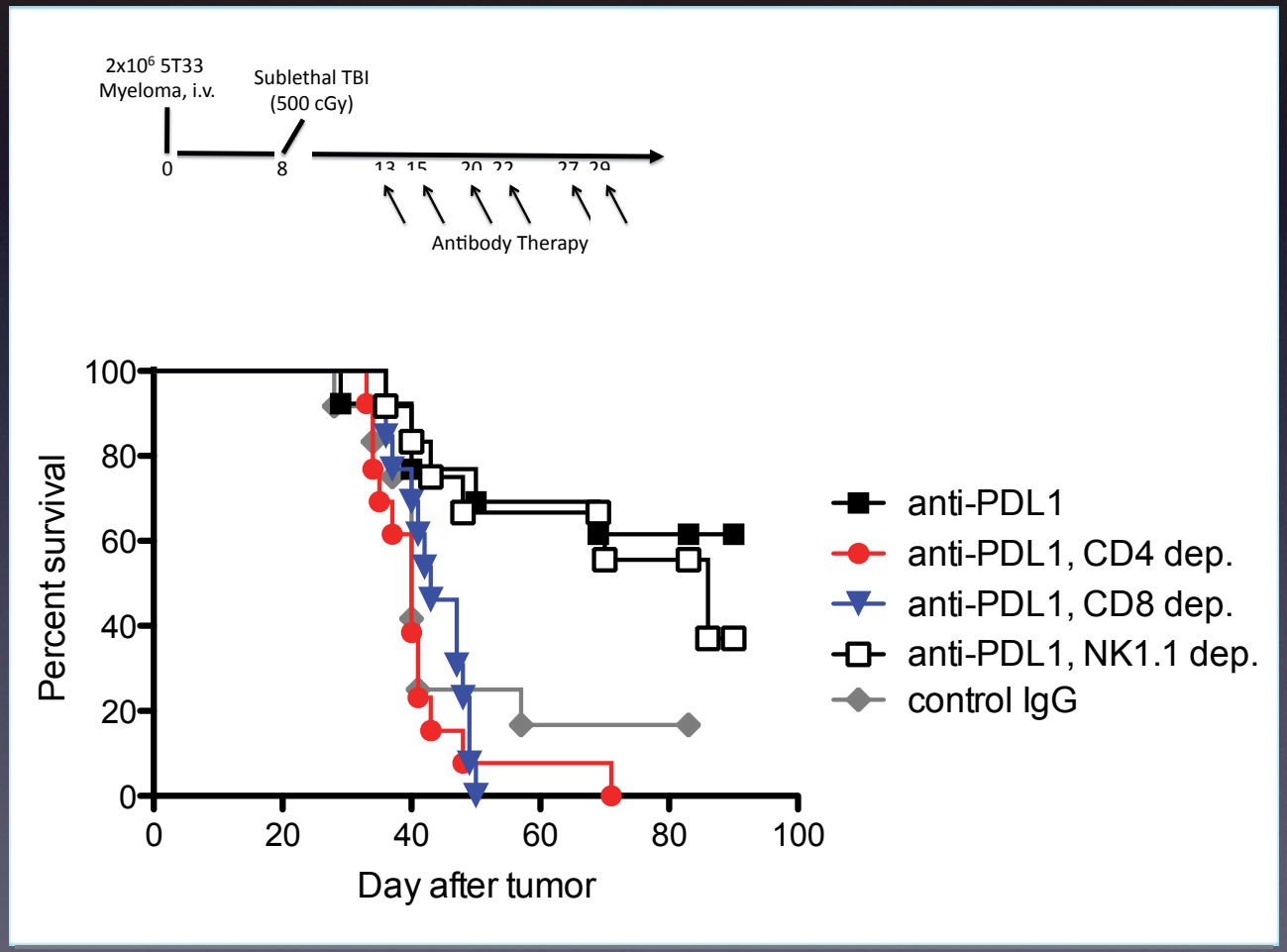
No previously published data showing that lymphodepletion + PD-1/PD-L1 blockade can eliminate myeloma (or any other tumor).

Effect of Sublethal TBI on Numbers of Cells in the Spleen and BM and Lymphocyte Subsets in the Spleen

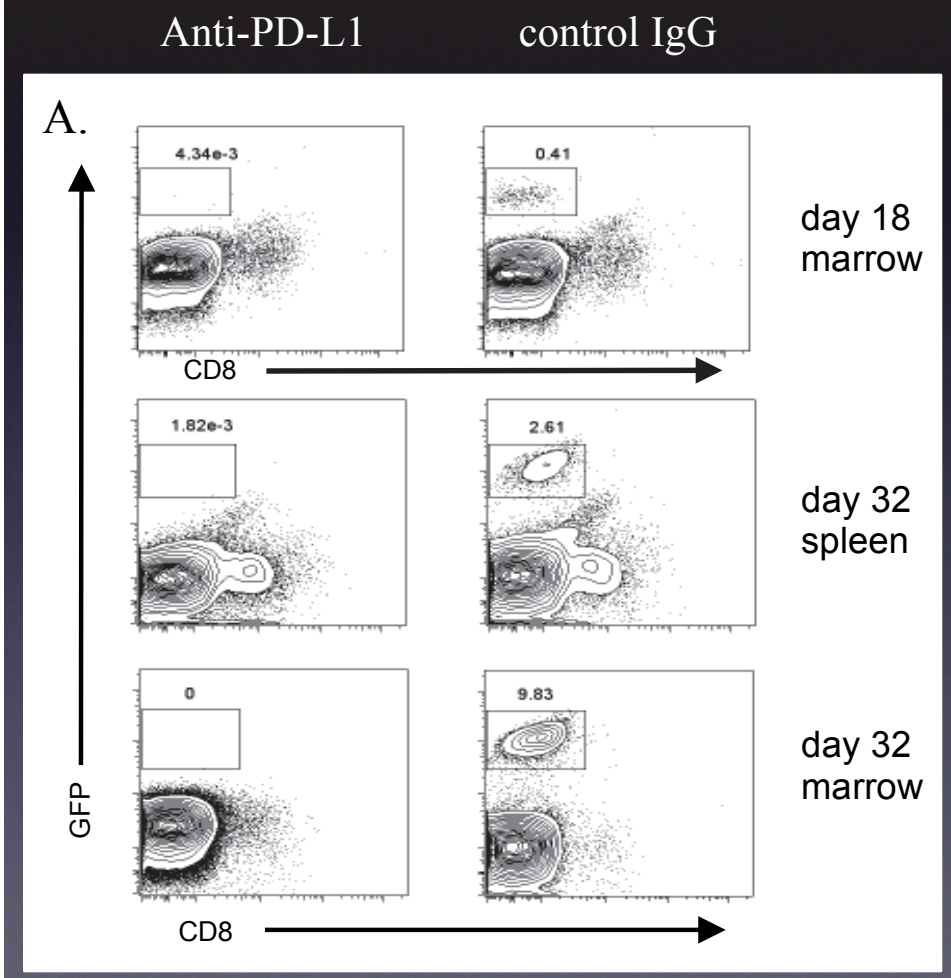


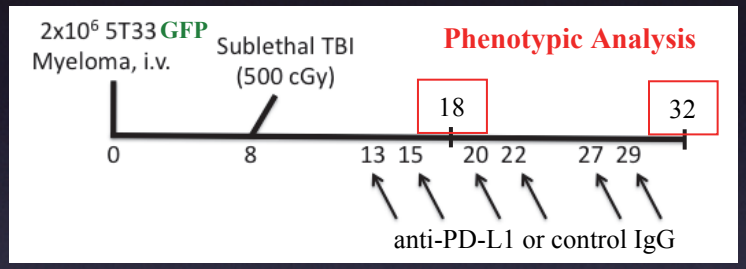
← Spleen only

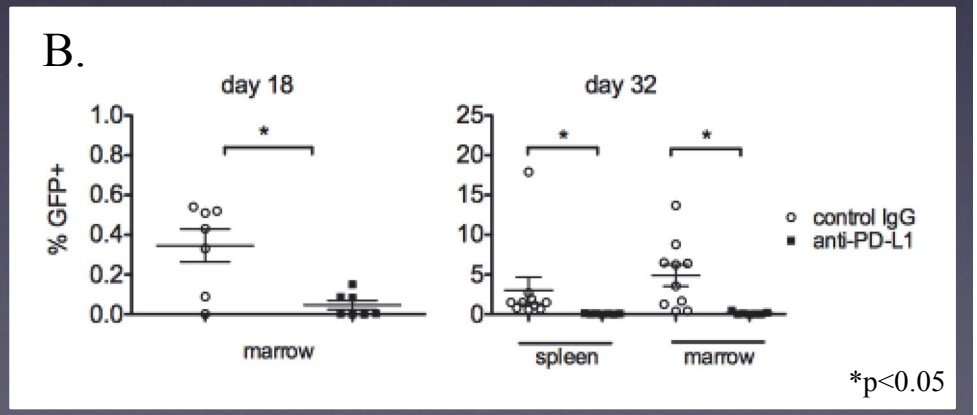
Both CD4 and CD8 T Cells Crucial to the Anti-Myeloma Effect of Sublethal TBI plus PD-1/PD-L1 Blockade



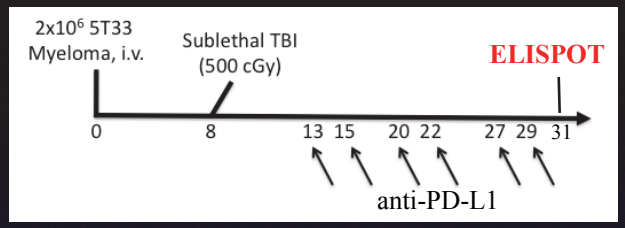
Elimination of Myeloma after Sublethal TBI and PD-1/PD-L1 Blockade Occurs Relatively Quickly

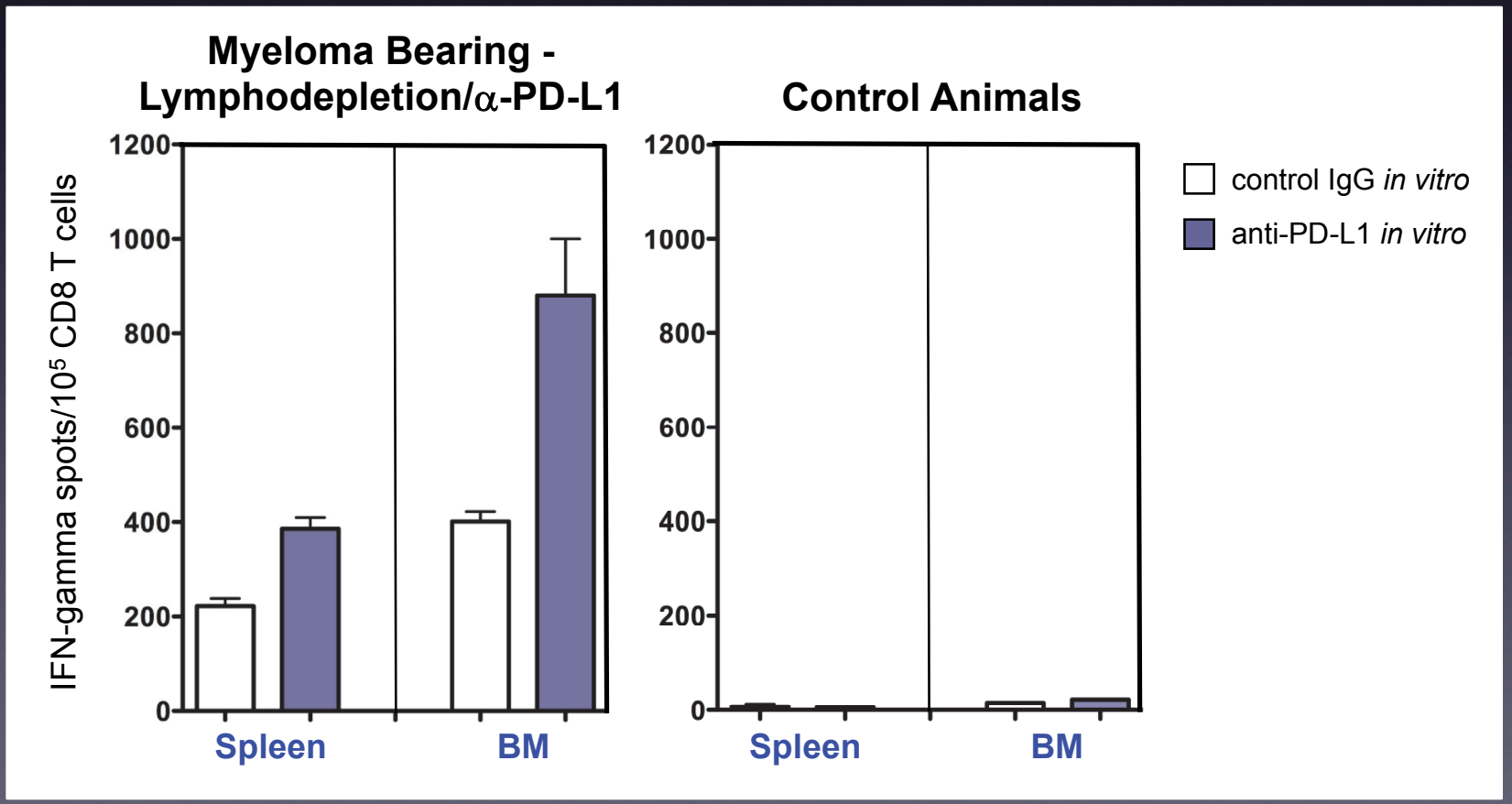




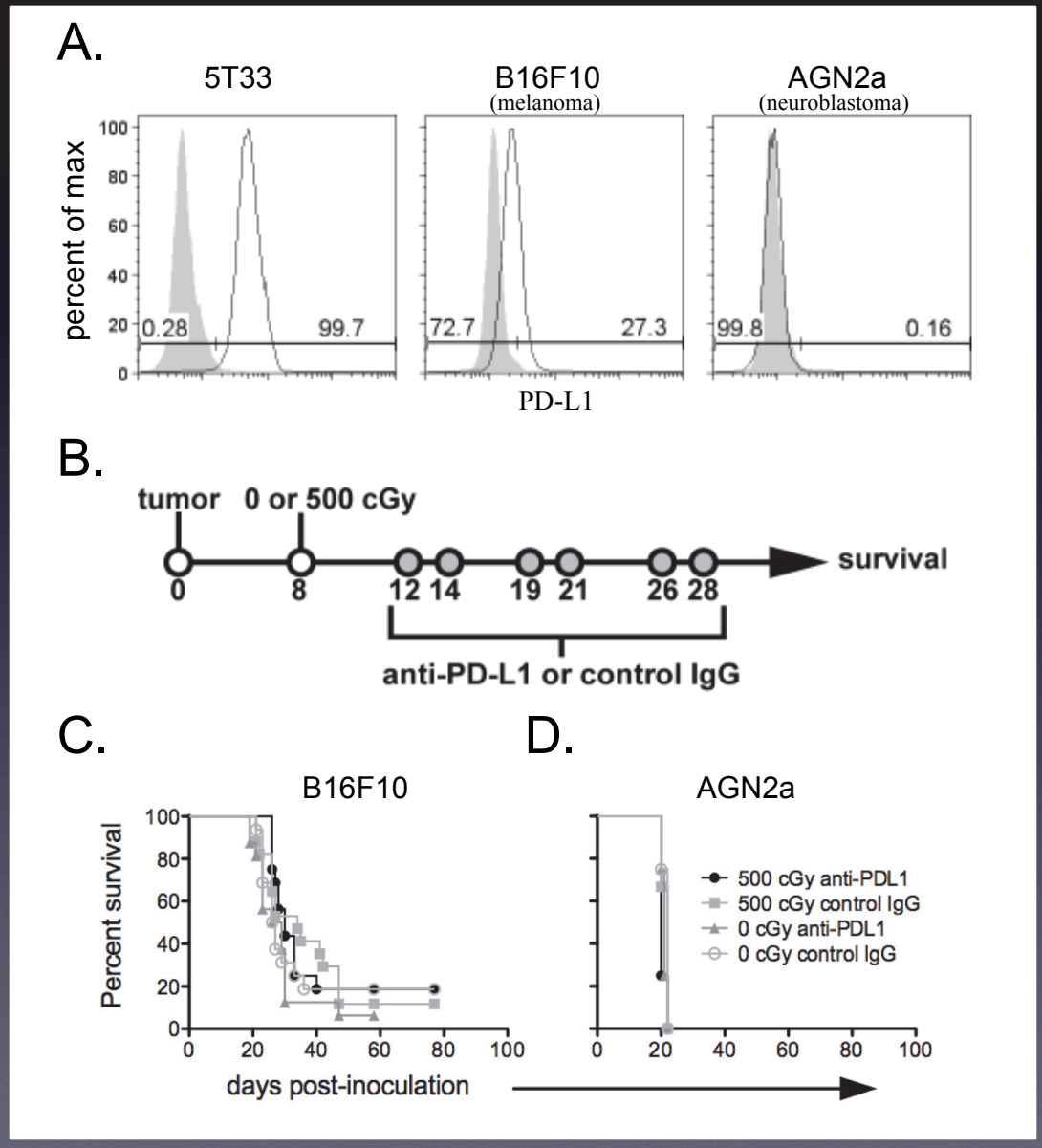


5T33 Myeloma-Reactive CD8 T Cells are Detected in the Spleens of Myeloma-Bearing Animals Treated with Lymphodepletion and anti-PD-L1





Sublethal TBI plus PD-1/PD-L1 Blockade has no Effect on Murine Melanoma or Neuroblastoma Tumor Growth



Summary

- The PD-1/PD-L1 pathway plays a major role in the suppression of immunity to myeloma.

- Nonmyeloablative (lymphodepleting) irradiation and PD-L1 blockade facilitates the rejection of established myeloma in preclinical studies, which represents a clinically translatable approach. This treatment combination could serve as a platform for other immune therapies (adoptive cell transfer, vaccines, etc.).

- Working Hypothesis: Lymphodepletion allows for the "reactivation" of myeloma-specific T cells that have been inactivated by PD-1/PD-L1 interactions. PD-L1 blockade prevents the re-inactivation of these T cells.

Questions & Issues to be Resolved

(lymphodepletion + PD-1/PD-L1 blockade)

1. *Is the anti-tumor effect of sublethal TBI + PD-L1 blockade limited to the 5T33 model or does it work in other hematologic malignancies?*

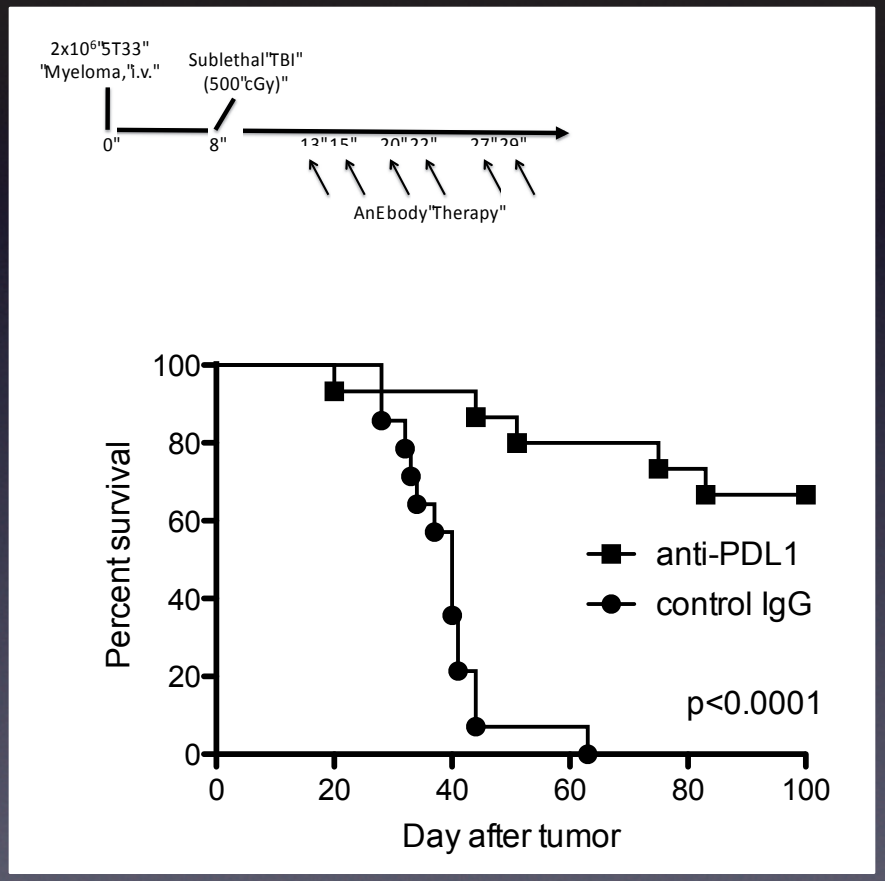
2. *Is the anti-myeloma effect due to the re-activation of "dysfunctional" PD-1^{+} T cells and/or the activation of new myeloma-reactive T cells?*

3. *How do CD4^{+} T cells contribute to the anti-myeloma effect?*

4. *What is the minimum dose of irradiation necessary to achieve the same anti-tumor effect? Can chemotherapy-based lymphodepletion mediate the same effect?*

5. *How long does PD-1/PD-L1 blockade need to occur?*

6. *The anti-myeloma effect is not 100%. Could the effect be enhanced with cellular therapy (or other therapies)?*



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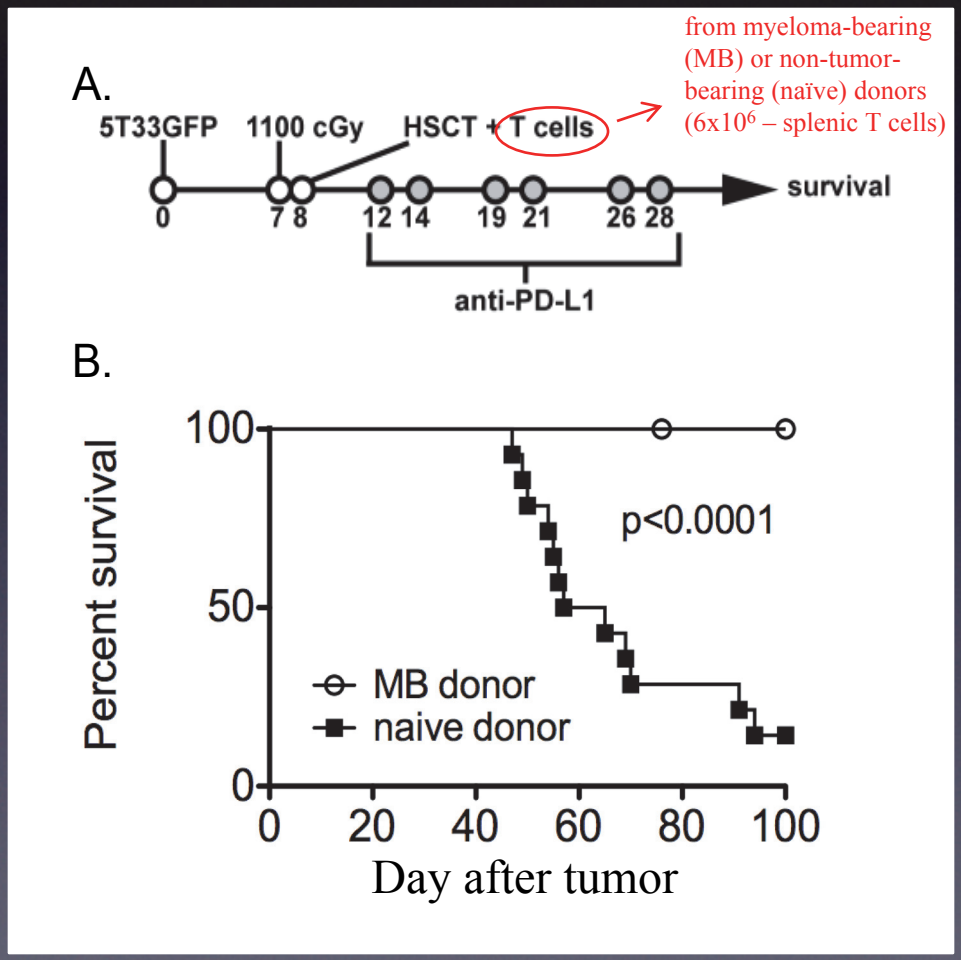
T Cells from Myeloma-Bearing Hosts Co-Transferred with BM Grafts are Effective at Eliminating Myeloma



T cells from myeloma-bearing donors came from the spleens of animals that had been injected with tumor cells 8 days prior to T cell isolation.