Prior to a patient receiving a dose of an adoptive cell transfer such as engineered autologous or allogeneic CAR-T cells, it is common to perform a lymphodepletion step often using high dose chemotherapy. This process is considered important to create sufficient space in the immune microenvironment, e.g. bone marrow, to allow the transferred cells to engraft. Further, it appears to elicit a favorable cytokine profile for establishment and proliferation of the donor lymphocytes. Previously, we have demonstrated the utility of anti-CD45 radiolabeled monotherapy (RT) using low non-myeloablative doses of 177Lu-CD45 antibody to effectively lymphodeplete in a targeted manner in experimental models prior to administration of adoptive cell therapy. Significantly, targeted conditioning with pan-CD45 RT, which selectively targets all nucleated immune cells, depletes not only lymphocytes, but also macrophages, as well as immune suppressive regulatory T cells (Tregs) and myeloid-derived suppressor cells in the immune microenvironment. It can also exert a direct anti-tumor effect on CD45+ hematopoietic cancers. In this study, we investigated an alternate payload for 177Lu-CD45, a 1 µB emitter (8.6 day half; 1.5 mm path length) for mediating effective lymphodepletion in mouse models. We performed preclinical studies using a 177Lu-labeled surrogate anti-mouse pan-CD45 antibody (30F11) to investigate in a mouse model the response of targeted RT lymphodepletion on particular immune cell types and resulting changes in immune cytokine expression. Following single dose administration of non-myeloablative doses of 177Lu-CD45-RT, peripheral blood, bone marrow and spleen samples were collected from 8-12 week C57Bl/6 mice at 96 hours and 10 days post-treatment for immunophenotyping to evaluate lymphodepletion and myeloid subsets for lymphodepletion, and serum for cytokine profiling. 177Lu-CD45-RT was shown to effectively lymphodeplete both lymphocyte and myeloid cells, inclusive of immune suppressive T reg subsets and MDSCs similar to 177Lu-CD45-RT. Studies evaluating this targeted lymphodepletion regimen in E.G7 lymphoma bearing mice prior to adoptive cell transfer with OVA-specific CD8+ T cells demonstrated enhanced anti-tumor response with both 131I and 177Lu-CD45 targeted lymphodepletion in comparison to adoptive cell therapy alone.

- 177Lu-labeled Anti-CD45 (177Lu-CD45) and 131I-labeled Anti-CD45 (131I-CD45):
  - The anti-mouse pan-CD45 antibody 30F11 was radiolabeled with 177Lu or 131I for use as a surrogate for radiolabeled pan-human-anti-granulocyte-macrophage stimulating factor (GMSF) to perform targeted lymphodepletion in mice.
  - Immune reactivity was confirmed in confirmed in CD45+ cell-binding assay to be > 95%.

For lymphodepletion studies in mice:
- Female adolescent C57Bl6 mice were treated with 80 µCi of 30F11 labeled with 10 or 40µCi of 177Lu or 50 or 100 µCi of 131I to determine the ability to selectively deplete immune cell subsets
- Immune cell subset quantification was measured by flow cytometry.

For lymphodepletion studies in OT I mouse model:
- Female adolescent C57Bl6 mice treated with injected unconventionally with OVA-expressing CD45+ E.G7-OVA lymphoma tumor cells until 100mm^3 tumor volume reached
- Approximately 7 days post tumor-cell injection, mice were treated with 177Lu-CD45 (40µCi), 131I-CD45 (100µCi), or received no lymphodepletion treatment
- Four days post-lymphodepletion, isolated CD3+ T cells isolated from CD45.2 OT I mice were administered to mice
- Tumor volume and body weight were monitored, and mice were sacrificed when tumor volume exceeded 2000 mm^3 or became necrotic.

Figure 1: Flow and antigen density analysis highlighting the considerable surface expression differential of CD45 between mature immune cell subsets and stem and progenitor cells.

![Image](image1.png)


Figure 2: Proposed mechanism of action of radiolabeled CD45 targeted lymphodepletion to support adoptive cell therapy. (1) targeted depletion of T and B lymphocytes creates a suitable immune homeostatic environment for incoming CAR-T cells; (2) depletion of immune suppressive cell populations that may hinder activation of CAR-T cells; (3) depletion of macrophages that may secrete cytokines implicated in CRS and neurotoxicity; and (4) potential anti-tumor effect on CD45+ blood cancer cells.

![Image](image2.png)

Figure 3: Anti-CD45 antibody was conjugated to DOTA at a ratio 20:1 and then labeled with 111In at a ratio of 5:1. C57Bl6 mice were injected i.p. with 60µg 111In-labeled anti-CD45 antibody with a specific activity of 5 µCi/µg and antibody distribution was monitored by microSPECT/CT. At indicated time points, CD45 antibody homed to immune system organs lymph nodes, spleen, and bone marrow.

![Image](image3.png)

Figure 4: Treatment of non-tumor bearing C57Bl6 mice with 20 or 40 µCi 177Lu-CD45 or B) 50 or 100 µCi 111In CD45 antibody was similarly effective in transiently lymphodepleting various immune cell populations without affecting bone marrow cells, red blood cells, or platelets.

![Image](image4.png)

Figure 5: Treatment of non-tumor bearing C57Bl6 mice with 40 µCi 177Lu-CD45 antibody was effective in transiently depleting various immune populations in the spleen including regulatory T cells (Treg).

![Image](image5.png)

Figure 6: Adoptive cell therapy of OT I CD8 T cells, with and without targeted lymphodepletion, in E.G7 syngeneic tumor model. Following establishment of E.G7 tumors, mice either received no treatment (Untreated) or lymphodepletion (OT I), or were conditioned with 40µCi 131I-CD45 or 100µCi of 131I-CD45 on Day 0. Mice (as described) then received 1 x 10⁶ OT I CD8+ 2 OVA reactive T cells on day 4. (A) Mean tumor volume or (B) individual mouse tumor volume indicated that 131I-CD45 and 177Lu-CD45-mediated targeted conditioning prior to adoptively transferred OT I T cells enabled enhanced control of E.G7 tumor growth, with a better response observed with 131I-CD45 pre-conditioning. OT I T cell persistence and expansion was confirmed in mice at the time of sacrifice. (C) Survival curve of mice on study. Tumor microenvironment analysis and characterization is in progress.

![Image](image6.png)

These studies demonstrate the feasibility in preclinical models of using a single low dose of 177Lu-CD45 or 111In-CD45 radiolabeled monotherapy as a transient non-myeloablative targeted lymphodepletion regimen prior to adoptive cell therapy.

11In-CD45 imaging demonstrated that CD45 targeting delivers radiation selectively to immune system organs.

Studies determined that 40 µCi 177Lu-CD45 or 100 µCi 111In-CD45 could effectively deplete various immune cell subsets in mice but spare bone marrow cells, red blood cells, and platelets.

In a model of adoptive cell therapy using CD45.1 OT I mice bearing EG.2-OVA tumors, mice that received 131I-CD45 RT-mediated lymphodepletion demonstrated enhanced tumor control over mice that did not receive lymphodepletion.

Lymphodepletion with 177Lu-CD45 resulted in greater tumor control than 111In-CD45 in this study.

This data supports development of 131I-CD45 targeted lymphodepletion prior to adoptive cell therapy using a single non-myeloablative dose of 74Se-DTPA or 131I-CD45 RT.