Poster #1420

SNMMI Annual Meeting
Anaheim, CA

June 22-25, 2019

Modeling Targeted Lymphodepletion With Radiolabeled CD45 antibody as a Preparative Regimen Prior to Adoptive Cell Therapy

Dale L. Ludwig[1], Wojciech Dawicki[2], Kevin J.H. Allen[2], Eileen M. Geoghegan[1], Ekaterina Dadachova[2]

[1] Actinium Pharmaceuticals, New York, NY, [2] University of Saskatchewan, SK, Canada

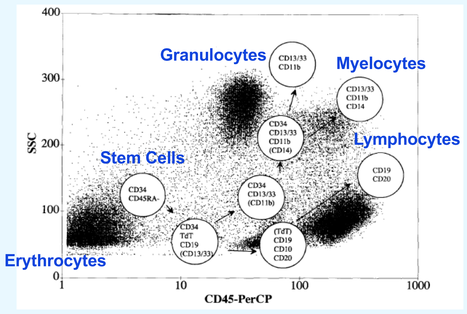
Abstract

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 radioimmunotherapy (RIT) using low non-myeloablative doses of ^{131}I-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 RIT, which selectively targets all nucleated immune cells, depletes not only lymphocytes, but also macrophages, as well as immune suppressive regulatory T cells (T-regs) 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 use of an alternate payload selection, specifically ^{177}Lu a beta emitter (6.6 day half life; 1.5 mm path length) for mediating effective lymphodepletion in mouse models. We performed preclinical studies using a ^{177}Lu-labeled surrogate anti-mouse pan-CD45 antibody (30F11) to investigate in a mouse model the response of targeted RIT lymphodepletion on particular immune cell types and resulting changes in immune cytokine expression. Following single dose administration of non-myeloablative doses of ^{177}Lu-CD45-RIT, 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 lymphoid and myeloid subsets for lymphodepletion, and serum for cytokine profiling. ^{177}Lu-CD45-RIT was shown to effectively lymphodeplete both lymphocyte and myeloid cells, inclusive of immune suppressive T regs and MDSCs similar to ^{131}I-CD45-RIT. Studies evaluating this targeted lymphodepletion regimen in E.G7 lymphoma tumor bearing mice prior to adoptive cell transfer with OVA-specific CD8+ T cells demonstrated enhanced anti-tumor response with both ^{131}I-CD45 and ^{177}Lu-CD45 targeted lymphodepletion in comparison to adoptive cell therapy alone.

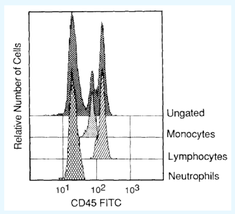
Methods

- ^{177}-Lutetium Anti-CD45 (^{177}Lu-CD45) and ^{131}I-Iodine Anti-CD45 (131I-CD45):
  - The anti-mouse pan-CD45 antibody 30F11 was radiolabeled with ^{177}Lu or ^{131}I for use as a surrogate for radiolabeled pan-human apamistamab (BC8) to perform targeted lymphodepletion in mice.
  - Immunoreactivity was confirmed in CD45+ cell-based binding assay to be > 95%.
- For lymphodepletion studies in mice:
  - Female adolescent C57Bl/6 mice were treated with 20ug of 30F11 labelled with 20 or 40μCi of ^{177}Lu or 50 or 100μCi of ^{131}I to determine the ability to selectively deplete immune cell subsets
  - Immune cell subset quantitation was measured by flow cytometry
- For lymphodepletion studies in OT I mouse model:
  - Female adolescent C57Bl/6 CD45.1 mice were injected subcutaneously 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 ^{177}Lu-CD45 (40μCi), ^{131}I-CD45 (100μCi), or received no lymphodepletion treatment
  - Four days post-lymphodepletion, isolated CD8+ 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 4000 mm3 or became necrotic

Figure 1: CD45 Is Differentially Expressed on Immune Cell Subsets



Adapted from Syrjälä et al. British Journal of Haematology (1994)



Loken et al. Cytometry (1990)

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

Conflict-of-Interest Disclosure: D.L.L. and E.M.G. have equity ownership in and are employed by Actinium Pharmaceuticals, Inc.; E.D. and W.D. receive research support from Actinium Pharmaceuticals, Inc.; K.A. has no disclosures.

Figure 2: Proposed Mechanism of Action for Radiolabeled CD45 Antibody Mediated Lymphodepletion Prior to Adoptive Cell Therapy

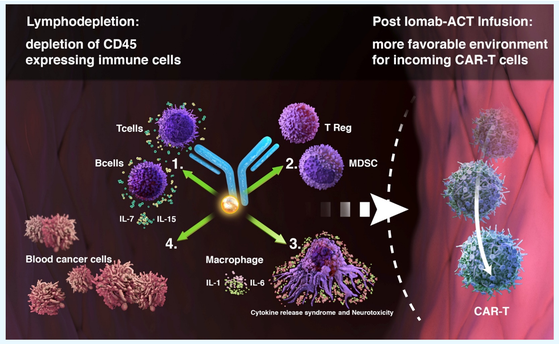


Figure 2: Proposed mechanism of action of radiolabeled CD45 targeted lymphodepletion to support adoptive cell therapy. (1) targeted depletion of T and B lymphocytes creates a suitable immune homeostatic environment for incoming CAR-T cells; (2) depletion of immune suppressive cell populations that may 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.

Figure 3: ^{111}In-Anti-CD45 Antibody Homes to Immune System Organs

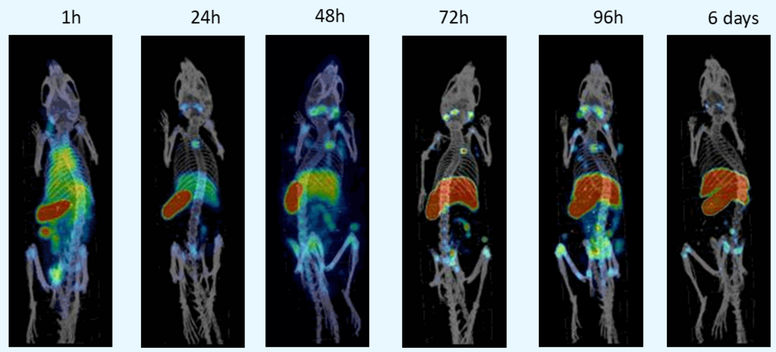


Figure 3: Anti-CD45 antibody was conjugated to DOTA at a ratio 20:1 and then labeled with ^{111}In at a ratio of 5:1. C57Bl/6 mice were injected i.p. with 60μg ^{111}In-labeled anti-CD45 antibody with a specific activity of 5 μCi/μg and antibody distribution was monitored by microSPECT/CT at indicated time points. CD45 antibody homed to immune system organs: lymph nodes, spleen, and bone marrow.

Figure 4: 177Lu-CD45 and131I-CD45 Transiently Deplete CD45+ Immune Cell Subsets Without Affecting Platelets, Red Blood Cells, or Bone Marrow Cells

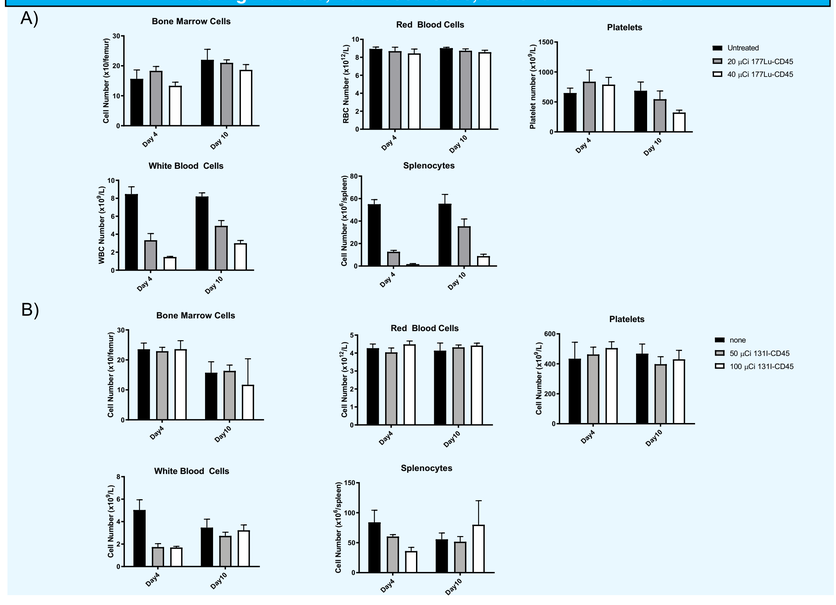


Figure 4: Treatment of non-tumor bearing C57B/6 mice with A) 20 or 40 μCi ^{177}Lu-CD45 or B) 50 or 100 μCi ^{131}-I CD45 antibody was similarly effective in transiently lymphodepleting various immune cell populations without affecting bone marrow cells, red blood cells, or platelets.

Figure 5: ^{177}Lu-CD45 Treatment Transiently Depletes CD45-Expressing Immune Cell Subsets in the Spleen

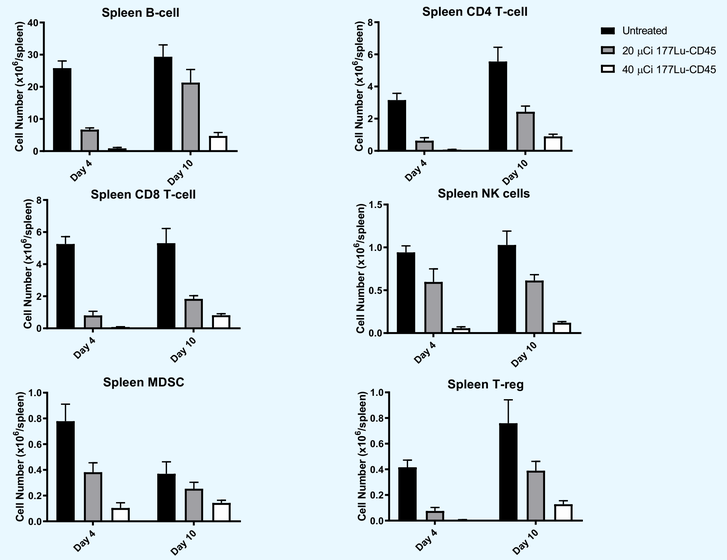


Figure 5: Treatment of non-tumor bearing C57B/6 mice with 40 μCi ^{177}Lu-CD45 antibody was effective in transiently depleting various immune populations in the spleen including regulatory T cells (T-regs).

Figure 6: ^{131}I-CD45 and ^{177}Lu-CD45 Lymphodepletion Enables Tumor Control in OT I Adoptive Cell Therapy Model

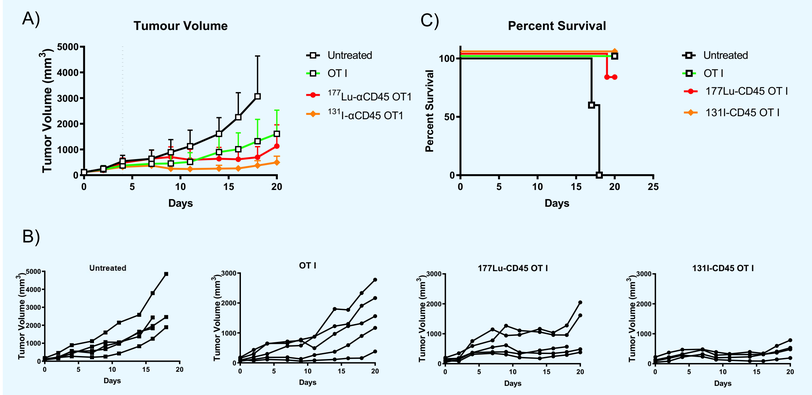


Figure 6: Adoptive cell therapy of OT1 CD8 T cells, with and without targeted lymphodepletion, in E.G7 syngeneic tumor model. Following establishment of E.G7 tumors, mice either received no treatment (Untreated), no lymphodepletion (OT I), or were conditioned with 40μCi ^{177}Lu-CD45 or 100μCi of ^{131}I-CD45 on Day 0. Mice (except Untreated) then received 1 x10^6 OT I CD8+ CD45.2 OVA reactive T cells on day 4. (A) Mean tumor volume or (B) individual mouse tumor volume indicated that ^{177}Lu-CD45 and ^{131}I-CD45-mediated targeted conditioning prior to adoptively transferred OT I T cells enabled enhanced control of EG.7 tumor growth, with a better response observed with ^{131}I-CD45 pre-conditioning. OT1 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.

Conclusions

- These studies demonstrate the feasibility in preclinical models of using a single low dose of ^{177}Lu-CD45 or ^{131}I-CD45 radioimmunotherapy as a transient non-myeloablative targeted lymphodepletion regimen prior to adoptive cell therapy
- ^{111}In-CD45 imaging demonstrated that CD45 targeting delivers radiation selectively to immune system organs
- Studies determined that 40 μCi ^{177}Lu-CD45 or 100 μCi ^{131}I-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 OT1 mice bearing EG.7-OVA tumors, mice that received CD45 RIT-mediated lymphodepletion demonstrated enhanced tumor control over mice that did not receive lymphodepletion.
- Lymphodepletion with ^{131}I-CD45 resulted in greater tumor control than ^{177}Lu-CD45 in this study.
- This data supports development of CD45 targeted lymphodepletion prior to adoptive cell therapy using a single non-myeloablative dose of ^{131}I-CD45 or ^{177}Lu-CD45 RIT.