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Lymphodepletion with CD45 Radioimmunotherapy as a Targeted Conditioning Regimen Prior to Adoptive Cell Therapy or CAR-T

Dale L. Ludwig, PhD[1], Wojciech Dawicki, PhD[2], Kevin J.H. Allen, PhD[2], Ravendra Garg, PhD[2], Eileen M. Geoghegan, PhD[1], Ekaterina Dadachova, PhD[2], Mark S. Berger, MD[1]

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

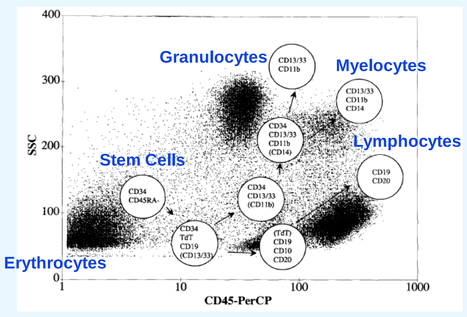
Abstract

Lymphodepletion, using chemotherapy such as fludarabine and cyclophosphamide (flu/cy), is recognized as a critical step to create a favorable immune homeostatic environment in patients prior to adoptive cell therapy (ACT) or CAR-T. However, flu/cy is a cytotoxic and non-specific regimen that some patients may not be able to tolerate and is also correlated with cytokine release syndrome (CRS) and possibly neurotoxicity that can occur following CAR-T administration. Targeted lymphodepletion with radioimmunotherapy (RIT) directed to CD45 may be a safer and more effective alternative to target and deplete immune cells, including immune suppressor cells and those implicated in CRS. It may also reduce tumor burden since CD45 is overexpressed on most types of leukemia and lymphoma. The CD45 antigen is found on all nucleated immune cells with increased expression on mature lymphoid and myeloid lineages. Anti-CD45 RIT, ^{131}I-apamistamab (BC8), is in a Phase III clinical trial as a myeloablative targeted conditioning regimen prior to allogeneic stem cell transplant in patients with active relapsed/refractory AML. Results from patients during dosimetry testing have shown that low non-myeloablative doses of ^{131}I-apamistamab were able to safely induce transient lymphopenia; low dose anti-CD45 RIT may therefore be a promising targeted modality to effectively lymphodeplete prior to ACT. Studies were performed with an ^{131}I-labeled anti-mouse CD45 antibody at varying non-myeloablative doses to investigate the effect of CD45-RIT targeted lymphodepletion on immune cell types and cytokine profiles. Following single dose administration of 50 to 200 µCi CD45-RIT, blood, spleen and bone marrow samples were collected from C57Bl/6 mice at 2 days and 4 days post-treatment for immunophenotyping and cytokine profiling. CD45-RIT was shown to mediate effective lymphodepletion of greater than 90% of lymphocytes (CD4 and CD8 T cells, CD19 B cells, and NK cells), but also CD4+, CD25+, FoxP3+ Tregs at doses that had negligible impact on bone marrow stem cells. Notably, in follow-up recovery studies, significant Treg suppression was sustained for at least 10 days post-lymphodepletion. CD45-RIT lymphodepletion also led to reductions in both MDSCs and other immune subsets. Concomitant with effective removal of cytokine sinks, levels of IFNg and IL-6 were unchanged in these non-tumor bearing mice. Although modest, trends for increased IL-15 levels in peripheral blood following targeted lymphodepletion were also observed. Results of CD45-RIT targeted conditioning prior to ACT in the E.G7/OT I animal model will also be presented. These results demonstrate that targeted lymphodepletion with CD45 RIT can be achieved in a safe and effective manner supporting advancement to clinical testing of a single, low-dose, outpatient regimen (Iomab-ACT) that may effectively replace flu/cy conditioning prior to CAR-T.

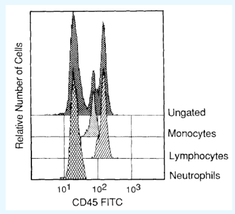
Methods

- 131-Iodine Anti-CD45 (131-I CD45): The anti-mouse pan-CD45 antibody 30F11 was radiolabeled with 131-iodine for use as a surrogate for pan-human CD45 131-I apamistamab (Iomab-ACT) 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 131-I CD45 (50-200µCi) to determine the appropriate dose required for transient lymphodepletion at varying time points (2-21 days)
  - Immune cell subset quantitation was measured by flow cytometry
  - Analysis was performed to determine the appropriate amount of antibody to be labeled from 20-100ug
  - Subsequent studies were performed with 20 ug of antibody labeled with 100 µCi 131-I CD45
- 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 131-I CD45
  - Four days post-lymphodepletion, activated CD8+ T cells isolated from CD45.2 OT I mice were administered to mice
  - Tumor volume and body weight were monitored, and blood and spleen were assessed for immune cell subsets and presence of engrafted CD45.2 OT I cells

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)

CD45+ Cell Type	CD45 Antigen Density (x10^3)
Monocytes	103 +/- 44
Lymphocytes	217 +/- 64
Neutrophils	36 +/- 16

Bikoue et al. Cytometry (1996)

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

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

Figure 2:Proposed Mechanism of Action for 131-CD45 Mediated Lymphodepletion Prior to Adoptive Cell Therapy

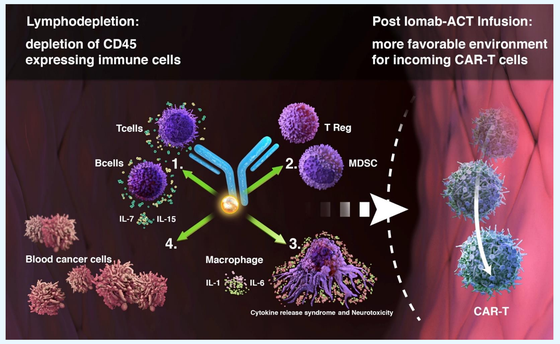


Figure 2: Proposed mechanism of action of 131-I CD45 RIT. (1) targeted lymphodepletion of lymphocytes to create suitable homeostatic environment for incoming CAR-T cells; (2) depletion of immune suppressive cell populations that may hinder activation of CAR-T cells; (3) depletion of macrophages that may secrete cytokines implicated in CRS and neurotoxicity; and (4) potential anti-tumor effect on CD45+ blood cancer cells.

Figure 3: 111In-Anti-CD45 Antibody Homes to Immune Cell Privileged Sites

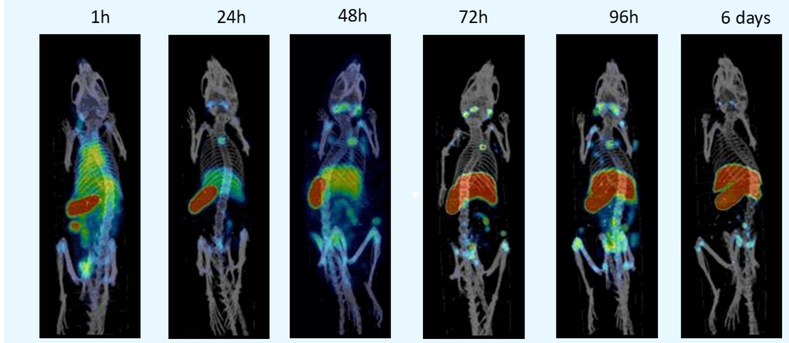


Figure 3: Anti-CD45 antibody was conjugated to DOTA at a ratio 20:1 and then labeled with 111In at a ratio of 5:1. C57Bl/6 mice were injected i.p. with 60ug 111In-labeled anti-CD45 antibody with a specific activity of 5 uCi/ug and antibody distribution was monitored by microSPECT/CT at indicated time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

Figure 4: 131-CD45 Lymphodepletion Preserves Bone Marrow Cells, Platelets, and Red Blood Cells but Depletes Lymphocytes, Splenocytes, and Myeloid Derived Cells

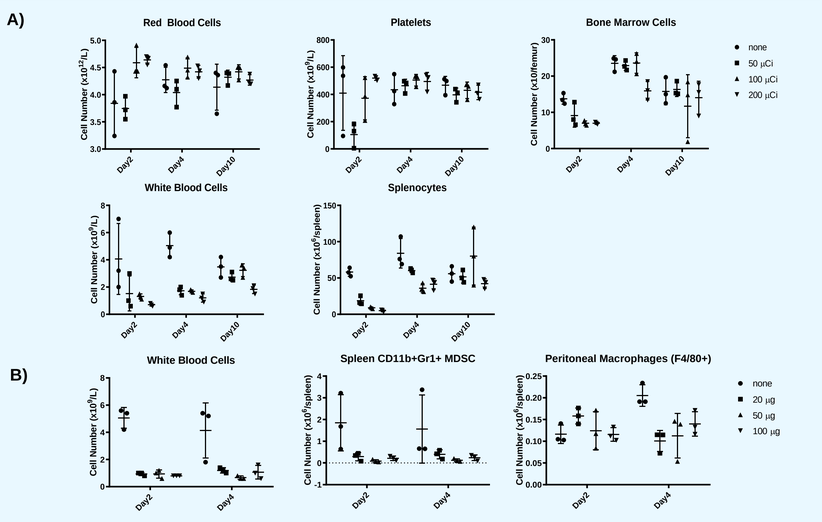


Figure 4: A) Dose finding studies were performed using 50-200 µCi to determine the appropriate dose of 131-I needed to define a non-myeloablative dose to safely lymphodeplete and (B) to determine the appropriate amount of antibody for labeling from 20-100µg. Based on these results, 20 ug of antibody was labeled with 100 µCi 131-I for targeted lymphodepletion.

Figure 5: 131-CD45 Treatment Transiently Depletes CD45-Expressing Immune Cell Subsets

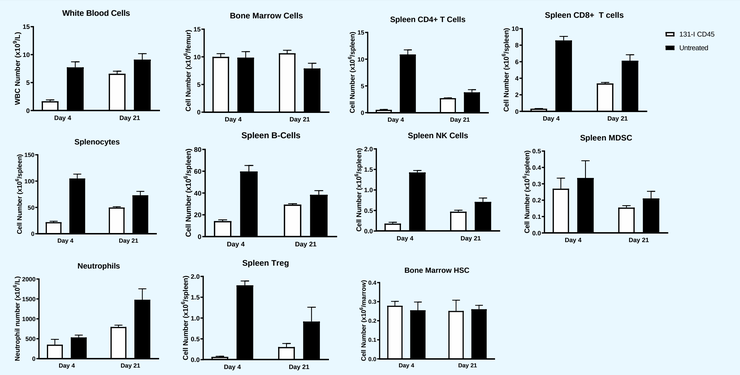


Figure 5: Treatment of non-tumor bearing C57B/6 mice with 100 µCi 131-I CD45 antibody was effective in transiently lymphodepleting various lymphocyte populations including T regs at a dose that does not impact bone marrow HSCs.

Figure 6: 131-CD45 Lymphodepletion Effect on Cytokines

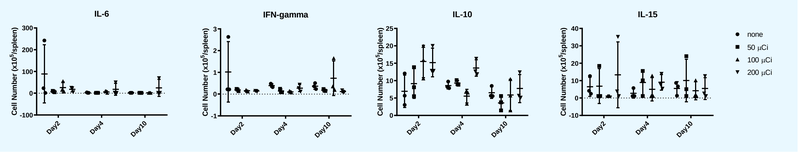


Figure 6: Cytokine profiles following CD45 targeted lymphodepletion of non-tumor bearing mice showed no impact on IL6 or IFN-g. Modest elevations of IL10 and a slight trend for an increase in IL15 were noted. Testing was performed by Mesoscale.

Figure 7: 131-I CD45 Lymphodepletion Safely Depletes Immune Cells in OT I Tumor Model

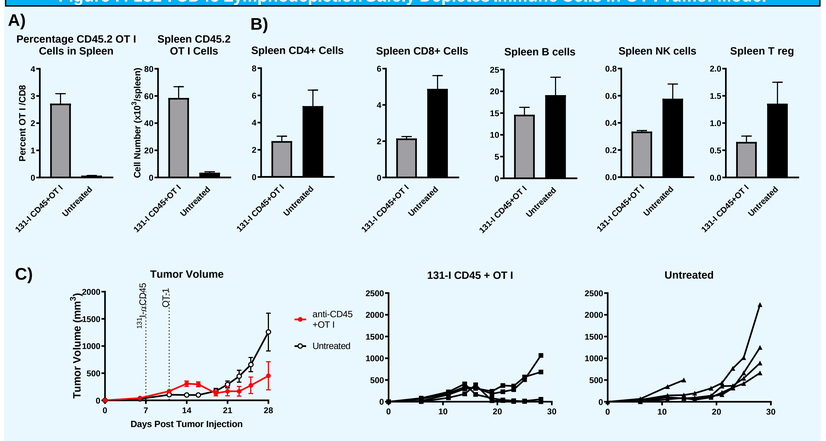


Figure 7: Following E.G7 tumor engraftment, mice were conditioned with 100µCi of 131-I CD45 and received 1 x10^6 OT I CD8 CD45.2 OVA reactive T cells on day 4. A) Targeted lymphodepletion with 131-I CD45 antibody enables engraftment and persistence of adoptively transferred CD45.2 OT I CD8+ T cells in the spleen 17 days post injection. B) Similar to conditioning in non-tumor bearing mice, 131-I CD45 lymphodepletion mediated decreases in multiple lymphoid cell subsets. C) 131-I CD45 mediated targeted conditioning and adoptively transferred OT I T cells enabled control of EG.7 tumor growth. Control mice received no conditioning or T cells. In test group, 2/4 mice achieved CR; in control group, 0/5 mice achieved CR. N = 4-5 per group

Conclusions

- These studies demonstrate the feasibility of using a low dose of 131-I CD45 radioimmunotherapy as a transient non-myeloablative targeted lymphodepletion regimen prior to adoptive cell therapy
- 111In-CD45 imaging demonstrated that CD45 targeting delivers radiation selectively to immune privileged tissues
- Studies determined that 100 µCi of 131-I CD45 could effectively deplete various lymphoid and myeloid cell subsets in mice but spare bone marrow cells, red blood cells, and platelets
- Immune cell depletion by 131-I CD45 targeted lymphodepletion is transient, with recovery evident by day 21 post-conditioning
- In a model of adoptive cell therapy using CD45.1 OT1 mice bearing EG.7-OVA tumors, transferred tumor-specific CD45.2 OT I T cells persisted in mice following administration 4 days post-131-I CD45 lymphodepletion and were able to control tumor cells compared to untreated mice.
- This data supports CD45 targeted lymphodepletion prior to adoptive cell therapy using a non-myeloablative dose of 131-I CD45 RIT.