Development of Radioimmunotherapy-Based Conditioning Regimens for Hematopoietic Cell Transplantation (HCT) Using Alpha-Emitters

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AML: *Non-Transplant* Outcomes in Patients > 60 Yrs (n=361)

![Graph showing survival rates for low, standard, and high risk patients](image)

- Low risk (n=25)
- Standard risk (n=208)
- High risk (n=128)
Chemo-Radiation Therapy

- Destroys diseased marrow
- Suppresses patient’s immune cells so that marrow graft will be accepted

Healthy Marrow Graft

- Replaces diseased marrow
Bone Marrow Harvest

PBSC Collection
Blood Vessel in Marrow

Cell Crossing Vessel Wall

# Diseases Treated by Marrow Grafts

<table>
<thead>
<tr>
<th>Nonmalignant</th>
<th>Malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aplastic Anemia</td>
<td>Leukemias</td>
</tr>
<tr>
<td>Genetic Diseases, e.g.</td>
<td>Lymphomas</td>
</tr>
<tr>
<td>Sickle Cell Disease</td>
<td>Myeloma</td>
</tr>
<tr>
<td>Thalassemia</td>
<td>Myelodysplastic Syndr.</td>
</tr>
<tr>
<td>Fanconi Anemia</td>
<td>Myelofibrosis</td>
</tr>
<tr>
<td>Immunodeficiencies</td>
<td></td>
</tr>
</tbody>
</table>
Survival

CML-CP: CY/ 12 Gy TBI

Years after Unrelated HCT

>51-55 yrs (n=13) 38%

40-50 yrs (n=39) 84%

<40 yrs (n=52) 84%

Aging and Hematopoietic Malignancies

The graph shows the adjusted incidence rate of various hematopoietic malignancies across different age groups. The malignancies include:
- AML (Acute Myeloid Leukemia)
- NHL (Non-Hodgkin Lymphoma)
- Myeloma
- CLL (Chronic Lymphocytic Leukemia)
- ALL (Acute Lymphoblastic Leukemia)
- CML (Chronic Myeloid Leukemia)

The incidence rate is highest in the age group of 5-9 years for AML and Myeloma, and decreases as age increases. For NHL and ALL, the incidence rate is relatively low across all age groups. CLL and CML show a gradual increase in incidence with age.
Nonmyeloablative Conditioning for Transplantation

- Allogeneic hematopoietic cell transplantation (HCT) is the only curative therapy for a variety of malignant and non-malignant hematological diseases
  
  *but* - high toxicity of conditioning ➔ high mortality
  - restricted to younger, medically fit patients

- Development of low dose regimen in a canine model:

  2 Gy TBI + posttransplant immunosuppression (MMF/CSP)

Clinical trials to treat elderly or medically infirm patients not eligible for conventional high dose HCT are ongoing
Survival

5-Year OS
CR1: 39%
CR2: 37%
Not in CR1/2: 24%
sAML: 26%

P=0.04

AML (n=274; Median Age 60; Includes MM)

- Not in CR1/2
- sAML
- CR2
- CR1

Years from HCT

Percent Relapse/progression

Gyurkocza, et al. JCO 28: 2859, 2010
Radioimmunotherapy in Hematopoietic Cell Transplantation

Aims:

- Replace high dose conditioning
- Target malignant cells more effectively
- Reduce toxicity of conditioning regimen (early and late)
  - Nonmalignant hematologic diseases
  - Medically infirm patients
- Target subsets of immune system
  - Increase specific immunosuppression
  - Less toxicity to other tissues
Advanced AML + MDS

$^{131}$I-anti-CD45 MAb / FLU / 2 Gy TBI

- 58 patients (22 related; 36 unrelated)
  - 47 Acute Myeloid Leukemia (AML)
    - 8 CR3
    - 20 Ref / Rel
    - 2nd
  - 11 high-risk myelodysplasia (MDS)
- Median age 63 (5–74) yrs

Pagel et al., Blood 114: 5444-5453, 2009
Advanced AML + MDS

$^{131}$I-anti-CD45 mAb / FLU / 2 Gy TBI
## Alpha Versus Beta Particles

<table>
<thead>
<tr>
<th>Isotope</th>
<th>$t_{\frac{1}{2}}$</th>
<th>Energy</th>
<th>Path length</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{131}$I</td>
<td>8 days</td>
<td>0.7 MeV</td>
<td>0.7 mm</td>
</tr>
<tr>
<td>$^{90}$Y</td>
<td>2.7 days</td>
<td>2.3 MeV</td>
<td>5 mm</td>
</tr>
<tr>
<td>$^{188}$Re</td>
<td>17 hours</td>
<td>1.1 MeV</td>
<td>4.4 mm</td>
</tr>
<tr>
<td>$^{213}$Bi</td>
<td>46 minutes</td>
<td>8.4 MeV</td>
<td>0.08 mm</td>
</tr>
<tr>
<td>$^{211}$At</td>
<td>7.21 hours</td>
<td>5.9 MeV</td>
<td>0.06 mm</td>
</tr>
</tbody>
</table>
**Characteristics of β-, α- emitters**

- **α-particle**
  - Range: 40-90 µM
  - LET 100keV/µM

- **β-particle**
  - Range: 400-7000 µM
  - LET 0.8 keV/µM
What Properties make Alpha-Emitting Radionuclides Attractive for Therapy?

• Specific alpha-emitting radionuclides may be well suited for targeted radiotherapy (Bi-212, Bi-213, At-211)

• Alpha-emitting radionuclides are very cytotoxic over short distances (i.e. radiation to individual cells)

• Alpha-emitting radionuclides offer high LET radiotherapy (i.e. cell repair is not possible)

• Alpha emission killing is independent of oxygen concentration

• Alpha emission killing is independent of dose rate
Radiolabeling Chemistry
Requirements

- A stable attachment of the radionuclide
- No alteration of the carrier molecule’s targeting
- Adequate specific activity
- Favorable secondary distribution of metabolites
"In the current donor crisis, we've had to be somewhat resourceful with your bone marrow transplant."
Canine Model for HCT: Anti-CD45 MAb (CA12.10C12, IgG₁)

CD45:
- Expressed on all hematopoietic cells
- 200,000 copies expressed on average leukocyte
- Required for antigen receptor-mediated activation of B- or T-cells
- Highly expressed on hematologic malignancies including leukemias and lymphomas
[I-123]anti-CD45 mAb Blood Clearance in Dog E510

% ID / g vs Time (hours)
Anti-CD45 MAb Levels in Dogs Treated with 0.4 - 0.6 mg/kg $^{213}$Bi-Anti-CD45 MAb
Comparison of Radioimmunotherapy and TBI

2 Gy TBI

3 Gy TBI
Nonmyeloablative BMT in Dogs with $^{213}$Bi-labeled Anti-CD45 MAb

- DLA-identical littermates

- 6 x $^{213}$Bi-anti-CD45 Conjugate
- 4 x $10^8$ cells/kg of BM
- MMF (Day 0-27)
- Cyclosporine (Day -1-35)
- Unlabeled MAb

Pathology

Sandmaier et al., Blood 100: 318, 2002
Bethge et al., Blood 101: 5068, 2003
Bethge et al., Transplantation 78: 352, 2004
DLA-identical Allograft After [Bi-213] CD45 MAb

Platelets

WBC

Granulocytes

Lymphocytes

[Bi-213] CD45 MAb
Days -3, -2

BM

MMF CSP

Weeks after Transplant

E885 Pre
E893 Donor

MNC

Whole MNC Gran

Blood, 2002
% Donor Chimerism in Dogs Transplanted with $^{213}$Bi-Anti-CD45 MAb

Microsatellite marker analysis of chimerism

DLA-identical allograft after [Bi-213] Anti-TCRαβ

Weeks 1 2 3 4 5 6 9 17 23 29 36 41 49
58 59 59

Blood, 2003
% Donor Chimerism in Dogs Transplanted with $^{213}$Bi-Anti-TCR$\alpha\beta$
Summary of $^{213}$Bi Studies

- Administration of radioimmunoconjugate safe
- Rapid and complete immune reconstitution
- All dogs treated with >1.5 mCi/kg achieved stable mixed chimerism
- Selective ablation of CD45+ cells or TCR$\alpha\beta+$ T-cells allows engraftment and replaces 2 Gy TBI
Rationale for Astatine in place of Bismuth

- $^{213}$Bi is not available in adequate doses for a scale-up in human patients
- Very high cost of producing $^{213}$Bi ($120,000-140,000 for $^{213}$Bi per patient)
- Very short half-life of $^{213}$Bi (45.6 min)
- $^{211}$At has a longer half-life (7.2 hours)

<table>
<thead>
<tr>
<th>Radionuclide</th>
<th>Type</th>
<th>Half-life</th>
<th>Path-length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astatine 211 ($^{211}$At)</td>
<td>α</td>
<td>7.2 hours</td>
<td>5.98</td>
</tr>
<tr>
<td>Bismuth 213 ($^{213}$Bi)</td>
<td>α</td>
<td>46 min</td>
<td>8.35</td>
</tr>
</tbody>
</table>
Comparison of Bi-213 and At-211

\(^{213}\)Bi and \(^{211}\)At-labeled anti-CD45 MAb (30F11) injection

- Toxicities
  - CBC
  - Liver enzymes
  - Renal function
- Biodistribution
- Pathological examination
Hepatic Toxicities

$^{213}$Bi Labeled 30F11

$^{211}$At Labeled 30F11

Normal BUN and creatinine

Nakamae et al., Cancer Res 69: 2408, 2009
Hematological Toxicities

$^{213}$Bi Labeled 30F11

$^{211}$At Labeled 30F11

Nakamae et al., Cancer Res 69: 2408, 2009
Marrow (10 uCi $^{211}\text{At} / 10 \text{ug 30F11}$)

Control   24 hr   48 hr

1 w   2 w   4 w

Nakamae et al., Cancer Res 69: 2408, 2009
Spleen (10 uCi $^{211}$At / 10 ug 30F11)

Control  

24 hr  

48 hr  

1 w  

2 w  

4 w  

Nakamae et al., Cancer Res 69: 2408, 2009
Spleen Weight after Injection of $^{211}$At-labeled anti-CD45 MAb (10µCi-10µg)

Graph showing the Grams of spleen weight over time after injection. The graph indicates that the weight drops to 25% of baseline 48 hours after injection.
Biodistribution of $^{211}$At-anti-CD45

Tissue distribution of $^{211}$At and $^{125}$I labeled anti-CD45 in a dog

Experimental setup:
Total dose of anti-CD45: 0.5 mg/kg
Necropsy @ 21.6 hours

Isotopes:
- $^{211}$At-anti-CD45
- $^{125}$I-anti-CD45
Biodistribution of $^{211}$At-anti-CD45

18% of cells in the liver are of hematopoietic origin
Dose Finding / Toxicity Studies with $^{211}$At-anti-CD45

Treatment scheme

No hematopoietic rescue

Day 0: treatment with $^{211}$At-anti-CD45
Total anti-CD45 dose: 0.5 mg/kg
Dose Finding / Toxicity Studies with $^{211}$At-anti-CD45

No hematopoietic rescue
Dose Finding / Toxicity Studies with $^{211}$At-anti-CD45

**Creatinine**
- Normal range: 0.6 - 1.6

**Bilirubin**
- Normal range: 0.0 - 0.5

**AST (GOT)**
- Normal range: 16-60

**Alkaline Phosphatase (ALP)**
- Normal range: 10-84 U/L
At-anti-CD45 as Conditioning in DLA-id HCT

- **CSP 15mg/kg BID PO**
  - Day -1 to +35

- **MMF 10mg/kg BID SC**
  - Day 0 to +27

- **Day 0: infusion of DLA identical bone marrow**

- **Day -3: treatment with 211At-anti-CD45**
  - Total anti-CD45 dose: 0.5 mg/kg

- **Day 365: necropsy**
At-anti-CD45 as Conditioning in DLA-id HCT

Median number of transfusions: 1 (range 1-2) (only for thrombocytopenia)
At-anti-CD45 as Conditioning in DLA-id HCT
211At-anti-CD45 as Conditioning in DLA-id HCT Chimerism

Granulocytes

Mononuclear cells

T-cells
$^{211}$At-anti-CD45 as Conditioning in DLA-id HCT

- **Aspartate aminotransferase**
  - Normal range: 16-60 U/l

- **Alkaline phosphatase**
  - Normal range: 10-84 U/l

- **Bilirubin**
  - Normal range: 0.0 - 0.5 mg/dl

- **Creatinine**
  - Normal range: 0.6 - 1.6 mg/dl
**211At-anti-CD45 as Conditioning in DLA-id HCT**

<table>
<thead>
<tr>
<th></th>
<th>H271</th>
<th>H388</th>
<th>H379</th>
<th>H251</th>
<th>H267</th>
<th>H232</th>
<th>H308</th>
<th>H349</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>211At dose (µCi/kg)</strong></td>
<td>630</td>
<td>540</td>
<td>520</td>
<td>410</td>
<td>360</td>
<td>290</td>
<td>200</td>
<td>155</td>
</tr>
<tr>
<td><strong>Marrow TNC infusion (×10⁸/kg)</strong></td>
<td>3.0</td>
<td>7.8</td>
<td>4.6</td>
<td>4.9</td>
<td>7.9</td>
<td>5.5</td>
<td>3.1</td>
<td>2.2</td>
</tr>
<tr>
<td><strong>Survival (weeks)</strong></td>
<td>48</td>
<td>49</td>
<td>40</td>
<td>52</td>
<td>52</td>
<td>53</td>
<td>52</td>
<td>18</td>
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<tr>
<td><strong>Cause of euthanasia</strong></td>
<td>End of study</td>
<td>End of study</td>
<td>End of study</td>
<td>End of study</td>
<td>End of study</td>
<td>End of study</td>
<td>End of study</td>
<td>Low donor chimerism</td>
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<tr>
<td><strong>Histopathology</strong></td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Decreased bone marrow cellularity</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mild regenerative liver hyperplasia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Thyroid hypercellularity</td>
<td></td>
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</tr>
</tbody>
</table>
211\textsuperscript{At} labeled Anti-TCR\textsubscript{αβ} as Conditioning

**Rationale for using anti-TCR\textsubscript{αβ}**

- Selective ablation of T-cells allows sustained low level donor engraftment
- Minimal toxicity due to limited distribution of the TCR\textsubscript{αβ}
- Suitable approach for treating non-malignant disease
Biodistribution of $^{211}$At-anti-TCR$_{\alpha\beta}$

**Experimental setup:**

- Total dose of anti-TCR$_{\alpha\beta}$: 0.15 mg/kg
- Necropsy @ 24 hours

**Isotopes:**

- $^{211}$At-anti-TCR$_{\alpha\beta}$
- $^{125}$I-anti-TCR$_{\alpha\beta}$
Dose Finding / Toxicity Studies with $^{211}$At-anti-TCR

**Treatment Scheme**

No hematopoietic rescue

- Day 0: treatment with $^{211}$At-anti-CD45
- Total anti-CD45 dose: 0.15 mg/kg

Day 365: necropsy
Lymphocyte Dynamics

Injection of 336 μCi/kg $^{211}$At-anti-TCR

% Cells in the lymphocyte gate

% Cells in the granulocyte gate

Granulocyte gate

Lymphocyte gate
Dose finding study: $^{211}\text{At-anti-TCR}_{\alpha\beta}$

$^{211}\text{At-anti-TCR}_{\alpha\beta}$ dose finding study

- **Granulocytes**
  - Median nadir 1500 cells/µL (773-3556)

- **Absolute lymphocyte count**
  - Median nadir 108 cells/µL (72-275)

- **Platelets**
  - Median nadir 84,000 cells/µL (69,200-149,000)

$^{211}\text{At-anti-CD45}$ dose finding study

- **Granulocytes**
  - Median nadir 9 cells/µL (1-298)

- **Absolute lymphocyte count**
  - Median nadir 60 cells/µL (34-272)

- **Platelets**
  - Median nadir 3000 cells/µL (1500-25,500)
# Dose Finding / Toxicity Studies with $^{211}$At-anti-TCR

<table>
<thead>
<tr>
<th></th>
<th>H430</th>
<th>H437</th>
<th>H439</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>$^{211}$At dose (µCi/kg)</strong></td>
<td>324</td>
<td>336</td>
<td>464</td>
</tr>
<tr>
<td><strong>Survival</strong></td>
<td>1 year</td>
<td>1 year</td>
<td>1 year</td>
</tr>
<tr>
<td><strong>Cause of euthanasia</strong></td>
<td>End of study</td>
<td>End of study</td>
<td>End study</td>
</tr>
<tr>
<td><strong>Histopathology</strong></td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
</tbody>
</table>
At-anti-TCR as Conditioning in DLA-id HCT

**Day 0**: infusion of DLA identical bone marrow

**Day -3**: treatment with $^{211}$At-anti-TCR

Total anti-CD45 dose: 0.15 mg/kg

**MMF 10mg/kg BID SC**

Day 0 to +27

**CSP 15mg/kg BID PO**

Day -1 to +35

**Day 365**: necropsy
Conclusions

- Infusion of $^{211}$At labeled anti-CD45 or anti-TCR Mab was well tolerated
- RIT with $^{211}$At labeled anti-CD45 could replace low dose TBI as conditioning in DLA-identical HCT
- Durable engraftment was in all dogs treated with >155 µCi/kg $^{211}$At-labeled anti-CD45
- No GVHD
- No clinical evidence of organ toxicity, incl. liver and kidney
- Mild elevations of liver function tests
- Subclinical hypothyroidism in 1 dog
211At-labeled MAb as Conditioning: Current and Future

- DLA-haploidentical HCT
- Canine lymphoma
- Canine models of non-malignant disease
  - Hematologic (Hemoglobinopathy)
  - Immune (SCID)
- Conditioning for gene therapy
- Tolerance induction for organ transplantation
- Nonhuman primate models of HIV
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