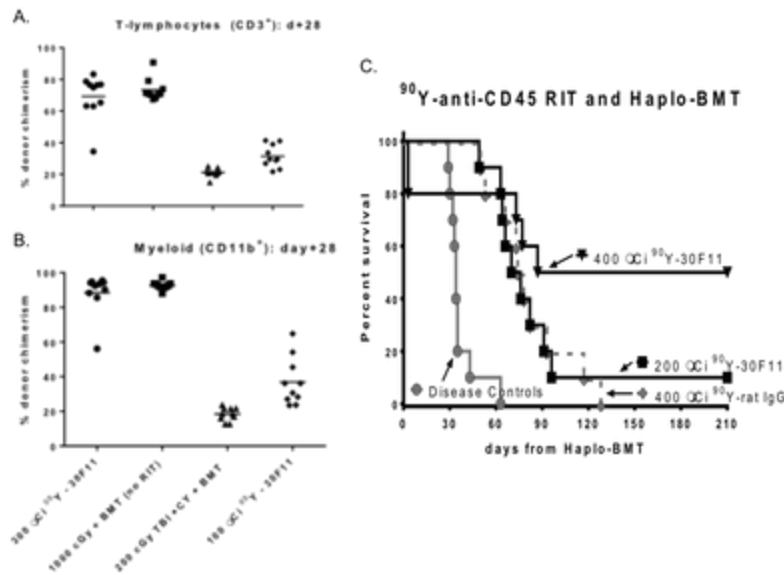


Make it work: marrow transplants from partially matched donors

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Extent of donor engraftment and overall survival following haploidentical HCT when conditioned with radioimmunotherapy and cyclophosphamide in murine leukemia model. Percent donor engraftment is equivalent when mice are conditioned with cyclophosphamide paired with either radioimmunotherapy or total body irradiation in multiple hematopoietic lineages, such as (A) T cells and (B) myeloid cells. (C) Conditioning mice with cyclophosphamide and radioimmunotherapy prior to HCT significantly increased overall survival compared to untreated leukemia bearing mice.

Figure provided by Dr. Johnnie Orozco

Hematopoietic cell transplantation (HCT) can provide lasting cures for patients suffering from leukemia, lymphoma, and myeloma. In particular, marrow transplants remain the most effective therapy for patients with advanced AML. Despite these successes there are many challenges still facing successful transplantation. Prior to transplantation the diseased marrow must be completely ablated otherwise patients risk chance of cancer recurrence, however, such treatments come at the cost of significant toxicity to patients. Common conditioning regimens include toxic chemotherapy and total body irradiation. If researchers can target these cytotoxic agents specifically to bone marrow, then the cancer cells are exposed to higher drug doses and healthy tissue remains intact.

Even with the perfect conditioning regimen many patients still face the challenge of finding a compatible marrow donor. The compatibility of a donor is determined by human leukocyte antigen (HLA) typing. HLAs encode components of the major histocompatibility complex (MHC), and while many HLA genes exist only 4 or 5 HLAs are evaluated for transplantation. Most clinical transplant centers require a donor to match 7/8 or 9/10 HLA alleles in order to be considered suitable. The most likely source for a match is a sibling as each sibling has a 25% chance of inheriting an identical complement of HLA alleles. However, 70% of patients fail to find a match within their family and

instead rely on the national marrow registry. This is particularly challenging for patients of ethnic minorities because their registration rates are lower and show greater attrition.

With both of these major challenges in mind, doctors and researchers at Fred Hutch and the SCCA are exploring radioimmunotherapy as a conditioning regimen for HCT using haploidentical donors in a mouse leukemia model. Dr. Johnnie Orozco and colleagues in the Clinical Research Division reported their findings in a recent issue of *Blood*. To better target the conditioning regimen Dr. Orozco used an antibody targeting CD45, a cell surface marker commonly expressed in leukemia and lymphoma cells. The antibody was conjugated to a radioactive isotope yttrium-90, enabling the radiation to be delivered directly to cancer cells, rather than exposing the entire body. To address the lack of perfect donor matches these studies were performed in mice where 50% of HLA markers are identical between donor and recipient. "We felt it was important to develop this novel conditioning approach using haploidentical donors, as not all patients that require a potentially curative bone marrow transplant may have a fully matched donor identified. This is especially challenging in patients from ethnic minority groups." said Dr. Orozco.

Conditioning regimens for HCT commonly include total body irradiation and a cytotoxic agent, fludarabine. Thus researchers compared the success of HCT engraftment replacing total body irradiation with radioimmunotherapy alone or in combination with fludarabine using haploidentical donors. Non-leukemic mice were treated with conditioning regimens and then analyzed 28 days later for engraftment success. Radioimmunotherapy alone was not sufficient for HCT, however, when paired with fludarabine the rate of engraftment was significant (~20%). With radioimmunotherapy functioning similar to total body irradiation, researchers also explored cyclophosphamide as an alternative to fludarabine. As a chemotherapy cyclophosphamide is similar to fludarabine, yet it better suppresses the immune system, which is important in the context of haploidentical donors. When analyzed at 28 days, engraftment levels were equivalent when radioimmunotherapy was paired with either fludarabine or cyclophosphamide. The researchers chose to move forward with cyclophosphamide as it's immunosuppressive activities were likely to provide long term benefits after HCT such as GVHD prophylaxis.

This combination was then tested in a mouse leukemia model, along with one important change to administration. In the initial studies HCT took place three days after conditioning, for the following studies this was extended to four days, with the concern that residual radiation could be affecting engraftment. This regimen resulted in highly successful HCT. The median survival of mice treated this way nearly doubled, and 50% of mice lived more than 200 days (compared to 34 days for control mice). Moreover, donor cells represented 80% of the marrow population from 28 days up to 6

months and beyond. Researchers also verified that donor cells were the major component of most differentiated cell types including T lymphocytes, B lymphocytes, and myeloid cells.

While these results demonstrate successful HCT using radioimmunotherapy, they did not formally test whether the new conditioning regimen reduced toxicity compared to total body irradiation, yet a decreased toxicity can be inferred, "we suspect a targeted approach to deliver radiation should be a better tolerated compared to total body irradiation. Specifically, mice treated with radioimmunotherapy did not experience significant weight loss or morbidity, compared to mice treated with total body irradiation. In addition, our targeted approach did not require fludarabine, eliminating a source of therapy related toxicity. Previously we have done [formal toxicity studies](#) evaluating the use of anti-CD45 radioimmunotherapy and found no significant renal or hepatic toxicity, but did find transient myelosuppressive effects that resolved within a few weeks, as would be expected from targeting CD45 hematopoietic tissues." said Dr. Orozco.

With these promising results, researchers at Fred Hutch are now exploring alternative radioisotopes for radioimmunotherapy. Using higher energy emitting atoms is likely to increase engraftment levels closer to 100%.

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[Orozco JJ, Kenoyer A, Balkin ER, Gooley TA, Hamlin DK, Wilbur DS, Hylarides MD, Frost SHL, Mawad R, O'Donnell P, Sandmaier BM, Fuchs EJ, Luznik L, Green DJ, Gopal AK, Press OW, Pagel JM.](#) 2016. Anti-CD45 radioimmunotherapy without TBI before transplantation facilitates persistent haploidentical donor engraftment. *Blood*. 127(3): 352-9.