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Early assessment of dosimetric and biological differences of total marrow irradiation versus total body irradiation in rodents

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ABSTRACT

Purpose: To develop a murine total marrow irradiation (TMI) model in comparison with the total body irradiation (TBI) model.

Materials and methods: Myeloablative TMI and TBI were administered in mice using a custom jig, and the dosimetric differences between TBI and TMI were evaluated. The early effects of TBI/TMI on bone marrow (BM) and organs were evaluated using histology, FDG-PET, and cytokine production. TMI and TBI with and without cyclophosphamide (Cy) were evaluated for donor cell engraftment and tissue damage early after allogeneic hematopoietic cell transplantation (HCT). Stromal derived factor-1 (SDF-1) expression was evaluated.

Results: TMI resulted in similar dose exposure to bone and 50% reduction in dose to bystander organs. BM histology was similar between the groups. In the non-HCT model, TMI mice had significantly less acute intestinal and lung injury compared to TBI. In the HCT model, recipients of TMI had significantly less acute intestinal injury and spleen GVHD, but increased early donor cell engraftment and BM:organ SDF-1 ratio compared to TBI recipients.

Conclusions: The expected BM damage was similar in both models, but the damage to other normal tissues was reduced by TMI. However, BM engraftment was improved in the TMI group compared to TBI, which may be due to enhanced production of SDF-1 in BM relative to other organs after TMI.

Total body irradiation (TBI) has been widely used as a standard component of the conditioning regimen for allogeneic hematopoietic cell transplantation (HCT) [1]. In addition to immunosuppression, TBI also provides a measure of leukemic control, especially in acute lymphoblastic leukemia (ALL) [2]. However, traditional TBI exposes un- or minimally involved vital organs, such as the gastrointestinal tract, lungs, eyes, liver, heart and kidneys to significant radiation, contributing to regimen-related toxicity and treatment-related mortality (TRM) [3,4]. Although a higher TBI radiation dose might overcome the increased risk of relapse, it also increases TRM, resulting in no overall survival benefit [5]. New conditioning strategies that simultaneously facilitate engraftment, provide leukemic control, and minimize organ toxicities are needed.

Given this unmet clinical need, a total marrow irradiation (TMI) protocol was developed and has been evaluated in clinical trials [6–8]. TMI focuses the field of delivered radiation to the bone marrow and other neoplastic foci while sparing non-target adjacent tissues, providing an enhanced therapeutic ratio (dose to sites of disease/dose to vital organs). Thus, it may be possible to increase the radiation dose to the sites of greatest disease burden while sparing less involved tissue to reduce overall pathologic effects. However, because of the lack of preclinical models, there is little understanding of the biological and mechanistic differences

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between TBI and TMI and their influence on bone marrow engraftment.

To address this gap in knowledge, we first developed a mouse model of TMI to test the hypothesis that TMI would induce less damage to organs but result in a similar marrow response compared to TBI. Then, we developed a clinically relevant TMI and chemotherapy transplant model to test the hypothesis that TMI would result in efficient engraftment while reducing risks of conditioning- and acute graft-versus-host disease-(GVHD)-mediated tissue damage. Furthermore, we analyzed underlying molecular mechanisms of those benefits of TMI by measuring the changes in stromal-derived factor 1 (SDF-1), a chemoattractant associated with successful engraftment [9], as well as epidermal growth factor (EGF) and amphiregulin (AREG), which are growth factors associated with wound healing responses.

Methods

Animal studies were approved by the Institutional Animal Care and Use Committee (IACUC #1106A00461) at the University of Minnesota. All rodents were kept in a standard vivarium and were fed a regular diet and water ad libitum.

Image guided TMI delivery system

A targeted irradiation jig (TIG) made of Styrofoam was built to deliver TBI and TMI treatments using an X-RAD 320 Biological X-ray Irradiator set up for large-field irradiations [10] (Fig. 1A). Radiation exposure time and use of portal film were optimized to distinguish the skeletal system, organs and placement of compensators (Fig. 1A ii). TBI was delivered with an open beam, whereas TMI was delivered by adding 2 mm copper compensators on the gut, lungs, and eyes, and placement was verified using XV films. In vivo dose verification was performed using thermoluminescent dosimeters (TLDs) and Gafchromic® EBT3 films. The dose reduction to vital organs was compared with that in our recent clinical TMI study. Further details of the experimental setup are described in Supplemental Methods.

TBI and TMI treatment effect in mice without HCT

BALB/c female mice (14–16 weeks old, Harlan Sprague–Dawley, Inc., IN) were divided into 3 groups (6–8 mice per group): no radiation, 8 Gy TBI, and 8 Gy TMI. All mice were anesthetized with an intraperitoneal injection of a ketamine (80 mg/kg)/xylazine (6 mg/kg) anesthetic combination before irradiation. Control mice (no irradiation) were similarly anesthetized. Two days after radiation, the bone marrow and small intestine were evaluated (Fig. 1B). Tissues were fixed, embedded in paraffin and evaluated semi-quantitatively for histological changes [11]. Tissue metabolic damage was evaluated in a subset of mice using longitudinal fluorodeoxyglucose (FDG) micro-PET/CT imaging following the method described previously [12,13].

Allogeneic HCT procedure

Male C57BL/6 mice (14–16 weeks old, Jackson) were used as recipients, and female BALB/c (8–10 weeks old, Jackson) were used as donors for the study (Fig. 1B). The recipient mice received either intraperitoneal phosphate buffered saline (PBS) or cyclophosphamide (Cy; Bristol Myers Squibb, Seattle, WA), 120 mg/kg per day as a conditioning regimen pre-HCT on days −2 and −1. At

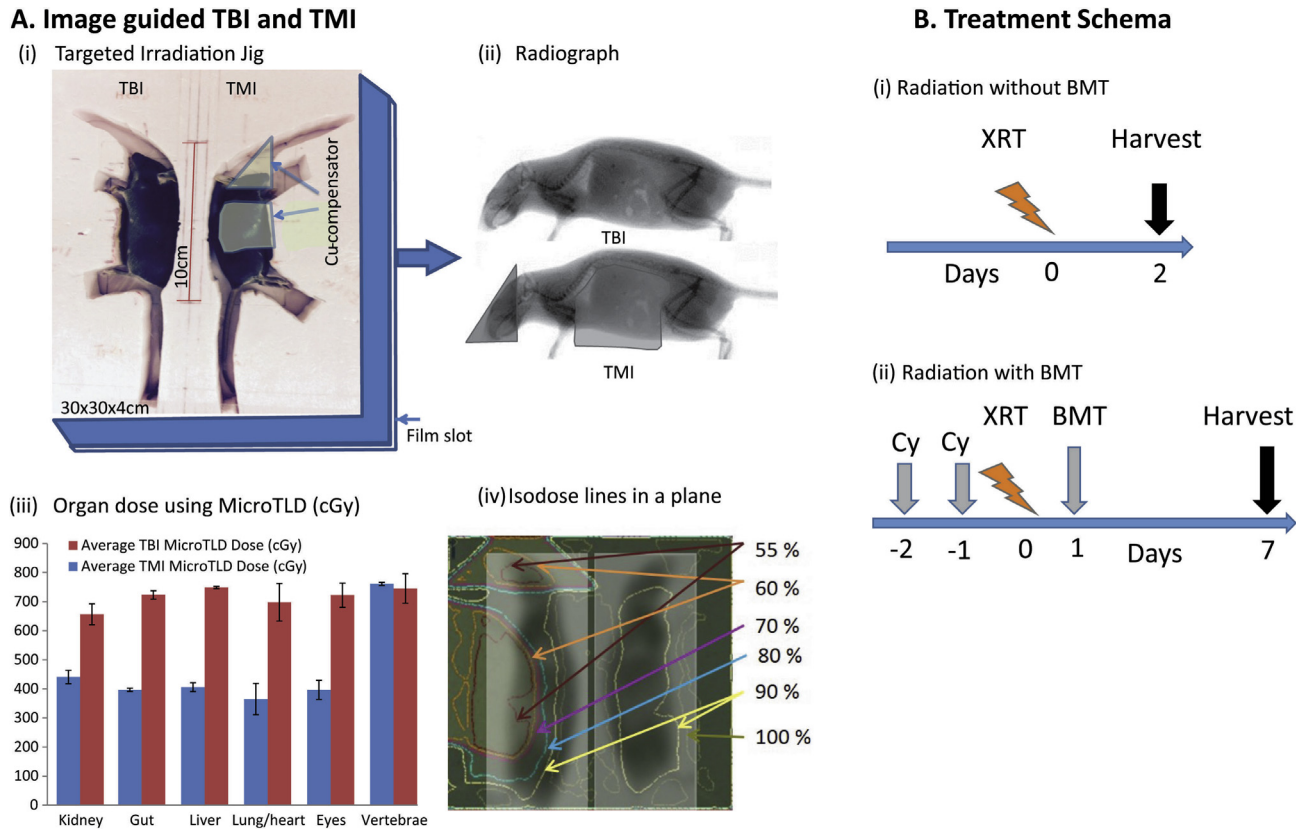

Fig. 1. Schema of image guided total marrow irradiation in mice. A. (i) Targeted Irradiation Jig with accessories, (ii) Radiograph of the mice with and without compensators in the jig, (iii) Organ dose measured using micro TLD, and (iv) isodose lines obtained from the Gafchromic film at the exit. B. Treatment Schema (i) Radiation without bone marrow transplant (BMT) and (ii) Radiation with BMT.