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Original Research

Irradiated Compared with Nonirradiated NSG Mice for the Development of a Human B-Cell Lymphoma Model

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NOD.Cg-*Prkdc^{scid} Il2rg^{tm1Wjl}*/SzJ (NSG) mice are a superior strain for the engraftment of human tumors, as they provide an ideal model to explore the potency, toxicity, and dosage of therapeutic drugs. Although whole-body nonlethal irradiation is often performed to enhance engraftment, the need for irradiation to establish a human B-cell lymphoma model using the NSG strain has not been addressed. In the current study, a mouse model of B-cell lymphoma was established by intravenous injection of human B-cell lymphoma Z138 cells into mice with and without irradiation. Tumor development, signs of engraftment, survivability of engrafted mice, histopathology, and immunohistochemistry were evaluated. Potential sex-associated variations in the model were assessed also. Irradiation of NSG mice did not enhance tumor cell engraftment, and nonirradiated animals had increased survivability. Mice with irradiation survived for a median of 27 d before being euthanized due to signs of morbidity, whereas those without irradiation had a median survival of 35 d. Both irradiated and nonirradiated mice were normal in activity until 3 wk after the injection of cells. At that time, the mice started to show signs of lymphoma including ruffled fur, decreased activity, and hindlimb paralysis. There were no significant differences in evaluated parameters between male and female mice. Therefore, we conclude that a model of B-cell lymphoma can successfully be established by using Z138 cells in nonirradiated male and female NSG mice.

Abbreviations: NHL, nonHodgkin lymphoma; NSG, NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ.

The National Cancer Institute defines nonHodgkin lymphoma (NHL) as cancer of lymphocytes, and it affects various organs of the immune system, including lymph nodes, spleen, and bone marrow. The several different forms of NHL include slow-progressing, fast-progressing, B-cell, and T-cell types.[6] Mantle cell lymphoma is a rare type of aggressive B-cell lymphoma (occurring in about 6% of lymphoma patients in the United States[2]) and is extremely difficult to treat. Patients with mantle cell lymphoma are treated with chemotherapeutic drugs, radiotherapy and transplantation of bone marrow,[7] but the lymphoma relapses after 3 to 4 y in nearly 50% of the patients.[4] Therefore, it is essential to develop strategies for enhancing the therapeutic options in patients with B-cell lymphoma and specific drugs that can cure the disease or prevent its relapse. Because animal experiments enable the preclinical testing of promising therapeutics for subsequent evaluation in humans, the development of an appropriate animal model is crucial.

Mice engrafted with human tumors act as a model for testing various therapeutic drugs for their potency, toxicity, and dosing.[1] Severe immunodeficient mice (SCID) mice have widely been used to disseminate tumor cells in vivo,[13] where the cells are engrafted via intravenous injection.[10,24] These mice have been used to develop a mouse model for human Burkitt lymphoma (a type of B-cell lymphoma) by using the Daudi cell line or SU-DHL-4 cells.[23] In these experiments, hindlimb paralysis and solid tumor development were observed as characteristic signs of lymphoma in the engrafted mice.[9,23] Whereas one group observed hindlimb paralysis even without irradiation of mice, the other did not see this development in any of their nonirradiated animals.[23] However, irradiation altered the pattern of tumor growth and the animals' responses to various chemotherapeutic drugs. It also led to variations in the animals' immune status in general and made them more susceptible to thyomas.[23] Therefore, whether to irradiate mice prior to the injection of B-cell lymphoma cells has been a topic of debate.

The development of NOD.Cg-*Prkdc^{scid} Il2rg^{tm1Wjl}*/SzJ (NSG) mice has provided a valuable tool for the development of a B-cell lymphoma model, because they lack mature B and T cells and various cytokines such as IL2, 4, 7, 9, 15, and 21, leading to impaired development of NK cells.[14,20] Some studies have shown that irradiating mice prior to the injection of various tumor cells enhances the engraftment and growth rate of the tumor,[3,21] but immunocompromised mice, especially NSG mice, are known to be sensitive to irradiation and subsequently may manifest increased morbidity and mortality.[12] In addition, the irradiation process can cause considerable distress in mice, and many institutions and IACUC require close monitoring and special care of mice after irradiation.[16] In our lab, mice routinely are provided

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with nutritional and fluid supplements and are placed on heating pads after irradiation, to prevent dehydration and death. Given irradiation's potential negative effect on animal health and the irradiation-associated variations reported in similar animal models, the elimination of irradiation may yield a less stressful and more reliable model for NHL.

The objective of the current study was to compare irradiated and nonirradiated NSG mice as a model for a specific type of B-cell NHL, mantle cell lymphoma. We evaluated engraftment, the development of clinical signs, and survival and potential sex-associated differences in each of those parameters in both irradiated and nonirradiated NSG mice injected with Z138 (mantle cell lymphoma) cells.

Materials and Methods

All animal experiments were performed in an AAALAC-accredited facility and approved by the City of Hope Beckman Research Institute IACUC.

Animals. NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG) mice (age, 4 to 8 wk) were obtained from an in-house breeding core and were derived from mice obtained from The Jackson Laboratory approximately 1 y prior to the current study. Mice were housed 4 per cage on corncob bedding (Bed-o'Cobs 1/4-in., The Andersons, Maumee, OH) in microisolation static cages (Allentown) covered with filter tops. Mice were designated SPF for Sendai virus, pneumonia virus of mice, mouse hepatitis virus, minute virus of mice, mouse parvovirus, mouse norovirus, Theiler murine encephalomyelitis virus, mouse reovirus type 3, mouse rotavirus, lymphocytic choriomeningitis virus, ectromelia virus, mouse adenovirus 1 and 2, mouse cytomegalovirus, polyoma virus, K virus, mouse thymic virus, Hantaan virus, Prospect Hill virus, cilia-associated respiratory bacillus, *E. cuniculi*, and *M. pulmonis*. They also were free of *Helicobacter* spp., Tyzzer disease virus, and endo- and ectoparasites. Mice were maintained on a 12:12-h light:dark cycle and given nesting pads and PVC tubes for environmental enrichment. Mice had free access to irradiated diet (Pico-Vac Lab Rodent Diet 5061, LabDiet, St Louis, MO) and reverse-osmosis–purified water in water bottles.

Cell culture. Z138 B cells were obtained from ATCC (Manassas, VA); these cells were infected with concentrated FUG2LW lentivirus, which expresses the genes for enhanced green fluorescence protein and firefly luciferase under the control of the *human ubiquitin* promoter over a range of multiplicities of infection from 0.1 to 1.3, as estimated by flow cytometry. The cells were cultured in RMPI 1640 media (Gibco BRL–Life Technologies, Invitrogen, Grand Island, NY) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and1 mM sodium pyruvate.

Experimental design. A preliminary experiment was performed to determine the optimal number of Z138 cells to inject for our model. Mice were injected via the tail vein with either 5×10^6 cells (10 male mice, 4 female mice) or 10×10^6 cells (4 male mice, 5 female mice) and observed for survivability and tumor engraftment (by bioluminescent imaging). The results of this preliminary experiment led us to use the dose of 5×10^6 cells for the remainder of the study.

Mice were divided randomly into 2 groups, which were irradiated ($n = 12$; 6 male mice, 6 female mice) or left nonirradiated ($n = 20$; 14 male mice, 6 female mice). Mice in the irradiated group were ^{137}Cs-irradiated at 150 rad 24 h before injection of cells. All mice were injected with 5×10^6 Z138 cells via the tail vein. All cages were placed overnight (approximately 12 h) on a hot-water blanket before being returned to their rack. Irradiated mice were offered HydroGel (Clear $H_2$0, Portland, ME) for 4 d after irradiation. To monitor engraftment, animals underwent bioluminescent imaging at various time points. In addition, mice were monitored for clinical signs of lymphoma (hunched posture, ruffled fur, decreased activity, hindlimb paralysis, and solid tumor development) and survival. They were euthanized when they exhibited signs of distress, hindlimb paralysis, inability to reach food or water, or a body condition score less than 2 (on a scale of 1 to 5).[22] In a subgroup of mice, blood was collected for flow cytometric analysis, and tissues were collected for histopathology and immunohistochemistry. In addition, because the experimental groups comprised both male and female mice, survival and engraftment in both sexes were analyzed.

Flow cytometric and luminometric analyses. Z138 cells infected with the FUG2LW (*Ub*^{Prom}*-eGFP-P2A-ffluc-WPRE*) lentivirus were analyzed for GFP expression by flow cytometry (Gallios Flow Cytometer, Beckman-Coulter, Fullerton, CA). Likewise, to measure infected Z138 cells for luciferase activity, cells were prepared by using the Luciferase Assay System (Promega, Madison, WI) and were analyzed by using the Veritas Microplate Luminometer (Turner Biosystems, Promega, Madison, WI). Whole blood was collected from 6 mice per group (3 male and 3 female mice from each group) at the end of the study, and flow cytometric analysis was performed by using antihuman CD45 conjugated with phycoerythrin and antimouse CD45 conjugated with allophycocyanin (Miltenyi Biotec, San Diego, CA) according to the manufacturer's protocol. Briefly, the collected whole blood was lysed by using 1× RBC lysis buffer (Sigma, St Louis, MO) and washed with PBS with 0.1% BSA. The antibodies were added and cells were washed again before flow cytometry (BD FACSCanto II, BD Biosciences, San Jose, CA) was performed.

Bioluminescence imaging. A subgroup of mice ($n = 4$ from each group) was imaged by using a Xenogen IVIS system every other day after tumor cell injection to determine when engraftment became visible. Once engraftment was visible in this subgroup, all other mice were imaged to confirm similar degrees of engraftment; and all mice then were imaged weekly thereafter until death. Briefly, mice were injected with D-luciferin (150 mg/kg IP; Promega) and anesthetized by using isoflurane. Mice were imaged at 5 min after the injection of D-luciferin to assess bioluminescence. The exposure time was 30 s, to obtain sufficient signal. Bioluminescence at day 12 was quantified by using Living image version 2.5 software (powered by Igor Pro 4.09A, Caliper Lifesciences, Hopkinton, MA).

Histopathology. Mice were euthanized by using CO_2, and tissues were collected from 3 animals per group for histopathology and immunohistochemistry. Liver, kidney, spleen, bone marrow, brain, and lung were collected in 10% formalin. Tissue sections were taken in paraffin blocks. Immunohistochemistry using an antiCD20 (Abcam, Cambridge, MA) antibody to stain Z138 cells (CD20 is expressed on the surface of Z138 cells) was performed on these cells.[15] All slides were counterstained with hematoxylin.

Statistical analysis. The Gehan–Breslow–Wilcoxon test was used for all statistical analyses of survival data. Two-tailed t tests of equal variance were used to analyze flow cytometric data. Bioluminescence were evaluated by using unpaired t tests. Statistical

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significance was defined as a P value of less than or equal to 0.05. All statistical analyses were done by using Prism 4 (GraphPad Software, San Diego, CA).

Results

Determining number of cells to be injected. Mice that received 10×10^6 cells survived for a median of 30 d, whereas mice injected with 5×10^6 cells survived for a median of 40 d (Figure 1) . Thus, the number of cells injected had a significant effect (P = 0.002) on the median survival time of mice. Because bioluminescence (engraftment) was not observed until day 12 in both groups, the survival of mice for only 30 d provided too short a period of time for any type of therapeutic trial. Therefore, we decided to inject 5×10^6 cells into the mice used for the remainder of the study.

Clinical signs. Both irradiated and nonirradiated mice were normal in activity and showed no signs of illness during the first 3 wk after injection. Mice showed signs of lethargy, ruffled fur, and decreased body condition during the fourth week after injection of the B-lymphoma (Z138) cells. Hindlimb paralysis occurred in approximately 25% of the mice in both irradiated and nonirradiated groups. Solid tumor formation was not observed.

Tumor cell engraftment. According to bioluminescence, both irradiated and nonirradiated mice showed engraftment by day 12 after tail-vein injection of the B-lymphoma cells (Figure 2). Irradiation did not improve progression of NHL in NSG mice, because both irradiated and nonirradiated groups engrafted similarly successfully (P = 0.99). Nonirradiated mice had an average bioluminescence of $17.77 \times 10^8 \pm 3.049 \times 10^8$ photons/s, whereas irradiated mice had an average of $17.82 \times 10^8 \pm 3.042 \times 10^8$ photons/s. All mice showed continued engraftment weekly thereafter (days 19 and 26) until death.

Survival. Median survival differed significantly (P = 0.001) between the irradiated and nonirradiated groups. Mice with irradiation survived for a median of 27 d before becoming moribund and being euthanized, whereas those without irradiation survived for a median of 35 d (Figure 3).

Flow cytometric analysis. Both irradiated and nonirradiated mice showed engraftment of tumor cells in the blood. We analyzed 6 mice from each group by flow cytometry using human CD45 antibody conjugated with phycoerythrin and mouse CD45 antibody conjugated with allophycocyanin. The population of tumor cells in irradiated mice ranged from 3% to 15% (mean, 5.45%; SE, 1.34%), whereas the nonirradiated mice had a tumor cell population of 2% to 20% (mean, 7.83%: SE, 3.37) in blood (P = 0.53; Figure 4).

Histopathology. There were no differences in staining characteristics between the irradiated and nonirradiated groups. Z138 cells were present in most of the organs sampled (Figures 5 and 6). Tumor cells were seen in large numbers in the bone marrow in both groups. Relatively low amounts of Z138 cells were noted in spleen, liver, and lung, and there were no signs of tumor cells in either the kidney or brain.

Sex-associated differences. In the preliminary study which evaluated the most appropriate cell concentration for the model, neither survival nor tumor engraftment differed by sex when mice were injected with either 5×10^6 or 10×10^6 Z138 cells. In mice injected with 5×10^6 cells, the median survival for both male (n = 10) and female (n = 4) mice was 35 d. Female mice (n = 5) injected with 10×10^6 cells survived for a median of 28 d, and

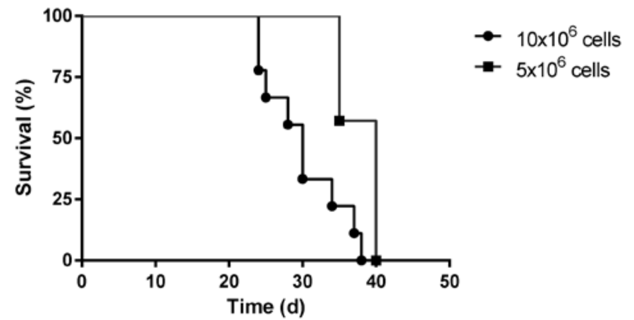

Figure 1. Survival of mice intravenously injected with 5×10^6 or 10×10^6 Z138 cells. The median survival time differed significantly (P = 0.002) between groups.

male mice (n = 4) survived for a median of 33 d (P = 0.179). Bioluminescent imaging showed engraftment in all mice at day 12 after injection.

Neither survival nor engraftment differed between male and female mice during the main experiment which compared irradiated and nonirradiated animals. The development of clinical signs was similar for both sexes, and engraftment was visible by 12 d after injection of cells. Both irradiated male (n = 6) and female (n = 6) mice survived for a median of 27 d. Nonirradiated male mice (n = 14) survived for a median of 34 d, whereas nonirradiated female mice (n = 6) survived for a median of 35 d (P = 0.23). In addition, histopathology and immunohistochemistry characteristics were similar between sexes. To evaluate tumor cell engraftment, we analyzed 3 mice of each sex in each group by flow cytometry. Irradiated male mice had an average tumor cell population of 5.69% (SE, 1.65%), whereas irradiated female mice had an average of 5.22% (SE, 2.48%) tumor cells (P = 0.88). Nonirradiated male mice had an average tumor cell population of 9.16% (SE, 6.96%), whereas nonirradiated female mice had an average of 6.51% (SE, 2.58%) tumor cells (P = 0.74).

Discussion

In this study, we validated a nonirradiated NSG mouse model of human B-cell NHL by using Z138 cells transduced with lentivirus carrying luciferase as a marker. Nonirradiated mice had similar clinical signs and engraftment characteristics when compared with those that underwent irradiation. The tumor cells were disseminated to various organs, including bone marrow, liver, and spleen, and nonirradiated mice survived for a median of 35 d, 1 wk longer than did the irradiated group. Postirradiation sickness can cause considerable morbidity, and our model eliminates the need for this potential stressor on the fragile NSG strain. The *Guide for Care and Use of Laboratory Animals*[11] states that the 3Rs (replacement, refinement, and reduction) principles must be considered by the IACUC and investigators when designing animal research studies and that "refinement refers to modifications of husbandry or experimental procedures to enhance animal wellbeing and minimize or eliminate pain and distress."[11] According to Public Health Services policy principle IV,[18] "proper use of animals, including avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices is imperative." Our study results show that the stress of irradiation can be eliminated in this model

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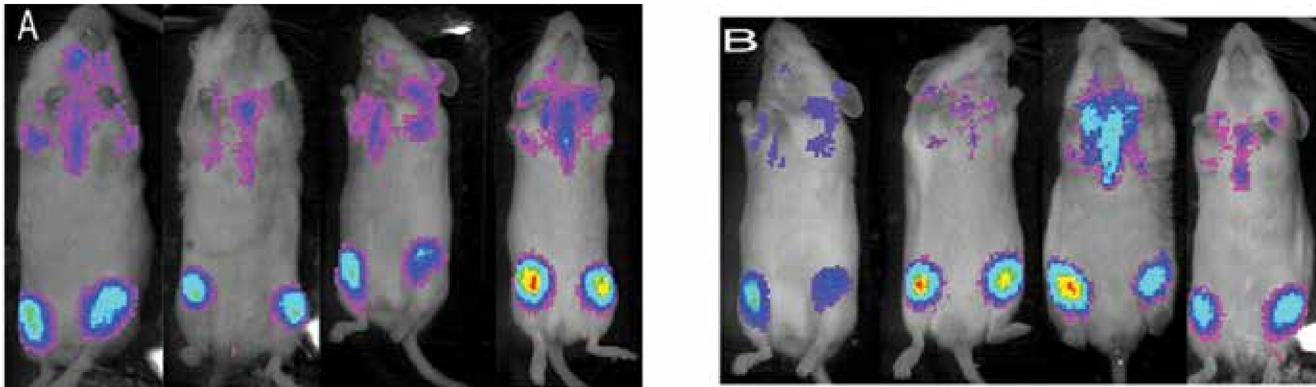

Figure 2. Bioluminescence imaging of mice at 12 d after injection of Z138 cells. (A) Irradiated mice. (B) Nonirradiated mice.

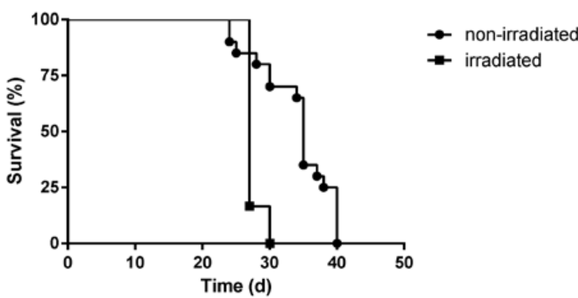

Figure 3. Survival of irradiated and nonirradiated mice engrafted with Z138 cells. The median survival time differed significantly (P = 0.001) between groups.

system without affecting tumor development. Therefore our NHL mouse model meets the goal of refinement mandated by the *Guide* and Public Health Services policy.

In a previous study, [23] injection of 5×10^6 compared with 10×10^6 Daudi cells in SCID mice did not affect the onset of the symptoms or survival of the mice. In our model using Z138 cells and NSG mice, there were no significant differences in the onset of engraftment of cells, but survival times differed between the 2 groups, with the lower tumor cell concentration resulting in a 10-d extension in median survival. This finding led us to conclude that a concentration of 5×10^6cells would be more appropriate for our NHL model, given that a longer survival time would support longer drug-trial periods. The median survival times of our irradiated (27 d) and nonirradiated (35 d) groups are shorter than those reported by others,[8] but the extension of survival time in the nonirradiated group mimics previous findings.[8]

Other colleagues[8,9] reported that irradiation of mice led to variations in the immune system leading to mixed results. In the SCID mice used in the cited studies,[8,9] irradiation rendered NK cells dysfunctional, leading to a hypothesized microenvironmental change in some of the organs and changing the pattern of tumor cell growth. The authors[8,9] reported inconsistency in the distribution of tumor cells in various tissues as well as variations in the development of hindlimb paralysis (and therefore of spinal cord invasion) between irradiated and nonirradiated groups. Our study results support this previous hypothesis, given that NSG mice lack functional NK cells, and we observed no differences in the distribution of tumor cells or development of clinical signs between irradiated and nonirradiated groups. Hindlimb paralysis has previously been reported to occur at different rates (from

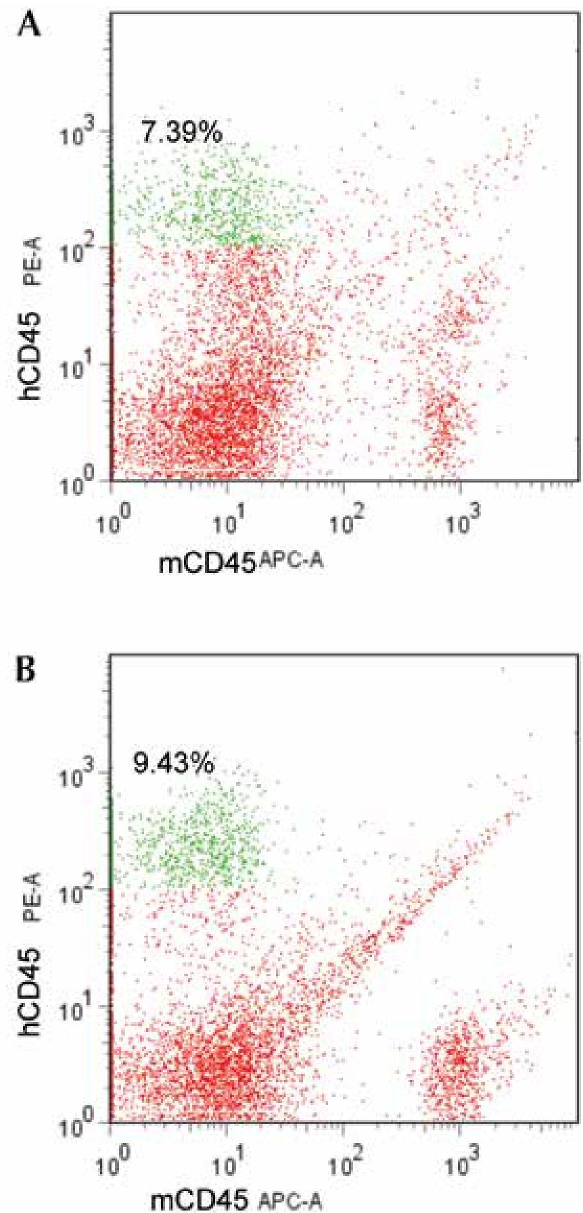

Figure 4. Flow cytometry of mouse blood. The green-labeled cell population (upper left corner of each panel) represents tumor cells (human Z138 cells). (A) Irradiated mice. (B) Nonirradiated mice.

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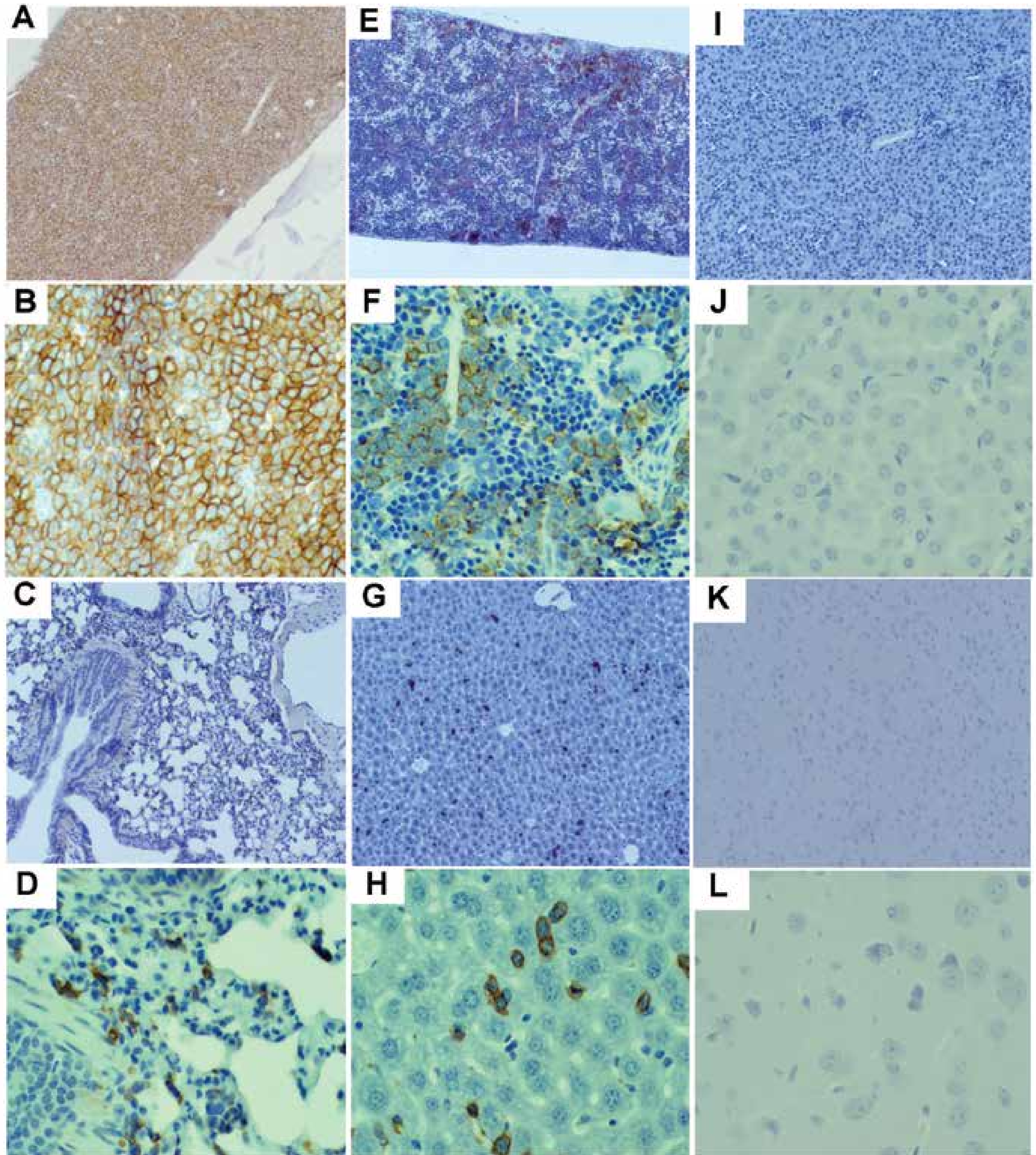

Figure 5. Engraftment of Z138 cells in organs of mice without prior irradiation. (A, B) Bone marrow. (C, D) Lung. (E, F) Spleen. (G,H) Liver. (I, J) Kidney. (K, L) Brain. CD20 immunohistochemistry; magnification: 10× (panels A, C, E, G, I, and K), 40× (panels B, D, F, H, J, and L).

0% to 100%) in other B-cell lymphoma models using various cell lines.[8,9,23,24] Hindlimb paralysis occurred in only approximately 25% of our mice.

The flow cytometric percentages of tumor cells in whole blood were fairly variable and were quite low (2% to 3%) in some mice. Subsequent studies in our laboratory have shown that flow cytometric analysis of the bone marrow gives a more accurate representation of tumor cell engraftment in this model (data not shown, but our experience shows the tumor cell population to be in the range of 30% to 60%). Although flow cytometry of bone marrow is not uncommon, blood is preferable that sample collection does not have to be an invasive or terminal procedure. Given

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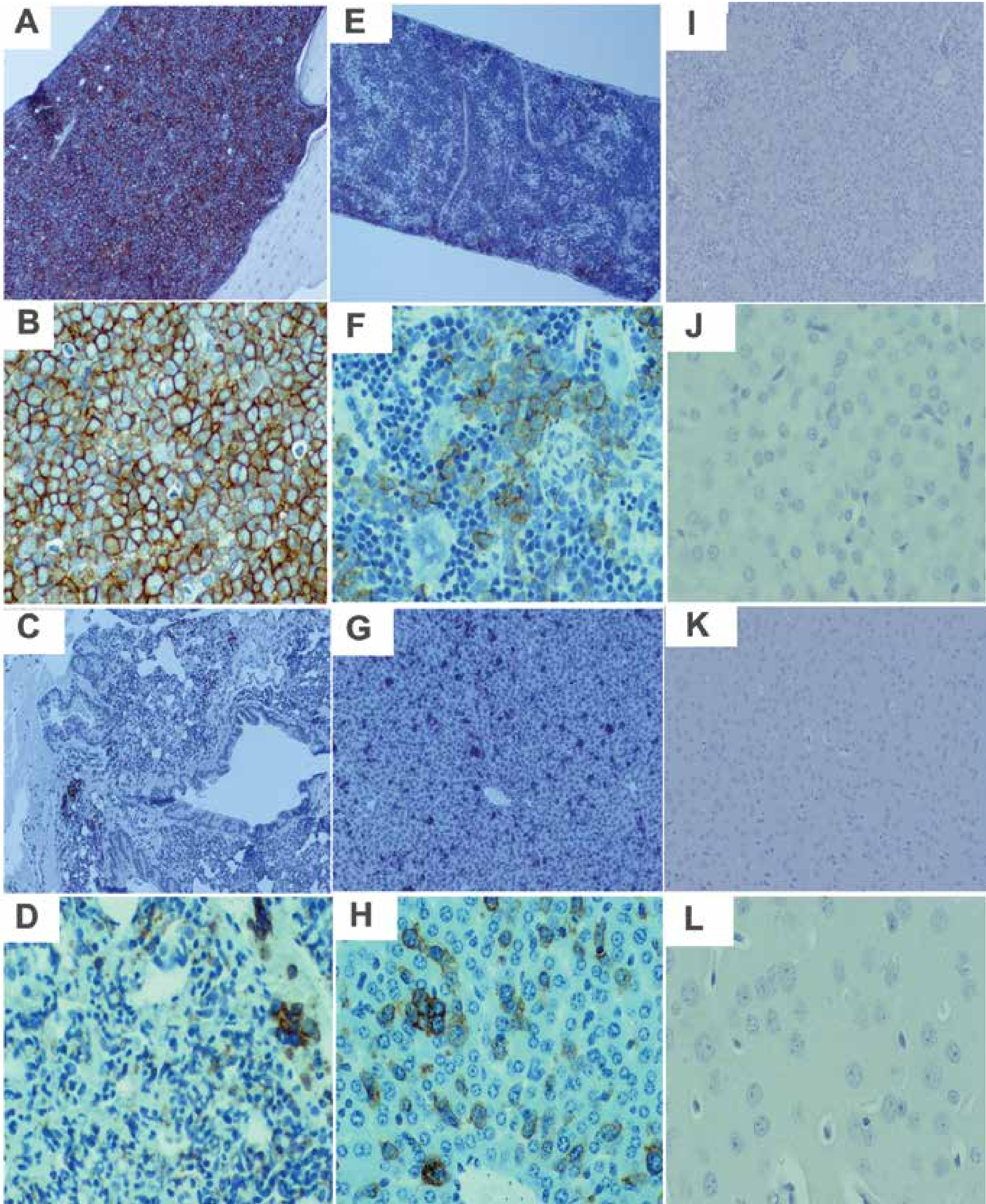

Figure 6. Engraftment of Z138 cells in organs of mice with prior irradiation. (A, B) Bone marrow. (C, D) Lung. (E, F) Spleen. (G,H) Liver. (I, J) Kidney. (K, L) Brain. CD20 immunohistochemistry; magnification: 10× (panels A, C, E, G, I, and K), 40× (panels B, D, F, H, J, and L).

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that the Z138 cells seem to hone almost exclusively to the bone marrow, our studies of various NHL treatments are now relying on measuring the tumor cell population in bone marrow, given that the whole blood measurements do not seem to accurately reflect the status of the animal.

Humans in advanced stages of NHL show increased involvement of the CNS and neurologic symptoms.[17] In our mouse model, we observed neurologic signs of hindlimb paralysis in approximately 25% of the mice with advanced disease. The prognosis for patients with CNS signs is very poor, with a median survival of 4 mo,[5] and this aspect of our model would enable research groups to study this specific component of disease progression. In addition, gastrointestinal tract symptoms are a common finding at the time of diagnosis of NHL, but the reason for the gastrointestinal tract tropism has not yet been understood.[19] Our mice lacked any gastrointestinal tract symptoms. It would have been advantageous to collect and histologically evaluate components of the gastrointestinal system, and this addition could be an area of future study.

In conclusion, our mouse model of B-cell NHL, which uses Z138 cells, does not require irradiation for engraftment of the tumor. Nonirradiated mice developed tumors and were normal in activity until 3 wk after injection of cells. Thereafter, mice gradually showed signs of lethargy, ruffled fur, and decreased activity, and a percentage developed hindlimb paralysis prior to euthanasia or spontaneous death. Male and female mice did not differ significantly in survival or tumor engraftment, and there were no differences in histopathologic or immunohistochemical features of various organs. Additional studies should be done with other human B-cell NHL cell lines (for example, Jeko, Granta) to evaluate whether irradiation of recipient mice is necessary to establish all crucial components of those models.

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References

1. **Brehm MA, Shultz LD, Greiner DL.** 2010. Humanized mouse models to study human diseases. Curr Opin Endocrinol Diabetes Obes **17:**120–125.
2. **Campo E, Raffeld M, Jaffe ES.** 1999. Mantle-cell lymphoma. Semin Hematol **36:**115–127.
3. **Cavacini LA, Giles-Komar J, Kennel M, Quinn A.** 1992. Effect of immunosuppressive therapy on cytolytic activity of immunodeficient mice: implications for xenogeneic transplantation. Cell Immunol **144:**296–310.
4. **Coiffier B.** 2005. Current strategies for the treatment of diffuse large B cell lymphoma. Curr Opin Hematol **12:**259–265.
5. **Ferrer A, Bosch F, Villamor N, Rozman M, Graus F, Gutierrez G, Mercadal S, Campo E, Rozman C, Lopez-Guillermo A, Montserrat E.** 2008. Central nervous system involvement in mantle cell lymphoma. Ann Oncol **19:**135–141.
6. **Friedberg JW, Fisher RI.** 2006. Diffuse large B-cell NHL. Cancer Treat Res **131:**121–140.
7. **Friedberg JW, Fisher RI.** 2008. Diffuse large B-cell lymphoma. Hematol Oncol Clin North Am **22:**941–952 [ix.].
8. **Ghetie MA, Gordon BE, Podar EM, Vitetta ES.** 1996. Effect of sublethal irradiation of SCID mice on growth of B-cell lymphoma xenografts and on efficacy of chemotherapy and/or immunotoxin therapy. Lab Anim Sci **46:**305–309.
9. **Ghetie MA, Podar EM, Gordon BE, Pantazis P, Uhr JW, Vitetta ES.** 1996. Combination immunotoxin treatment and chemotherapy in SCID mice with advanced, disseminated Daudi lymphoma. Int J Cancer **68:**93–96.
10. **Ghetie MA, Richardson J, Tucker T, Jones D, Uhr JW, Vitetta ES.** 1990. Disseminated or localized growth of a human B-cell tumor (Daudi) in SCID mice. Int J Cancer **45:**481–485.
11. **Institute for Laboratory Animal Research.** 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
12. **Kawata A, Han T, Dadey B, Weier HU, Okazaki M, Yokota S, Fukiage T, Xiao H, Block AM, Barcos M, Henderson E, Yoshida M, Seon BK.** 1993. Establishment and characterization of the tumors of chronic lymphocytic leukemia cell line in nude and SCID mice. Leuk Res **17:**883–894.
13. **Kawata A, Yoshida M, Okazaki M, Yokota S, Barcos M, Seon BK.** 1994. Establishment of new SCID and nude mouse models of human B leukemia–lymphoma and effective therapy of the tumors with immunotoxin and monoclonal antibody: marked difference between the SCID and nude mouse models in the antitumor efficacy of monoclonal antibody. Cancer Res **54:**2688–2694.
14. **Leonard JE, Johnson DE, Felsen RB, Tanney LE, Royston I, Dillman RO.** 1987. Establishment of a human B-cell tumor in athymic mice. Cancer Res **47:**2899–2902.
15. **Li Y, Williams ME, Cousar JB, Pawluczkowycz AW, Lindorfer MA, Taylor RP.** 2007. Rituximab–CD20 complexes are shaved from Z138 mantle cell lymphoma cells in intravenous and subcutaneous SCID mouse models. J Immunol **179:**4263–4271.
16. **Moccia KD, Olsen CH, Mitchell JM, Landauer MR.** 2010. Evaluation of hydration and nutritional gels as supportive care after total-body irradiation in mice (*Mus musculus*). J Am Assoc Lab Anim Sci **49:**323–328.
17. **Montserrat E, Bosch F, Lopez-Guillermo A, Graus F, Terol MJ, Campo E, Rozman C.** 1996. CNS involvement in mantle-cell lymphoma. J Clin Oncol **14:**941–944.
18. **Office of Laboratory Animal Welfare.** [Internet]. 2002. Public health service policy on humane care and use of laboratory animals. [Cited date 2 June 2013]. Available at: http://grants.nih.gov/grants/olaw/references/phspol.htm
19. **Romaguera JE, Medeiros LJ, Hagemeister FB, Fayad LE, Rodriguez MA, Pro B, Younes A, McLaughlin P, Goy A, Sarris AH, Dang NH, Samaniego F, Brown HM, Gagneja HK, Cabanillas F.** 2003. Frequency of gastrointestinal involvement and its clinical significance in mantle cell lymphoma. Cancer **97:**586–591.
20. **Shultz LD, Ishikawa F, Greiner DL.** 2007. Humanized mice in translational biomedical research. Nat Rev Immunol **7:**118–130.
21. **Silobricic V, Zietman AL, Ramsay JR, Suit HD, Sedlacek RS.** 1990. Residual immunity of athymic NCr/Sed nude mice and the xenotransplantation of human tumors. Int J Cancer **45:**325–333.
22. **Ullman-Cullere MH, Foltz CJ.** 1999. Body condition scoring: a rapid and accurate method for assessing health status in mice. Lab Anim Sci **49:**319–323.
23. **Yan JS, Chen XY, Li WP, Yang Y, Song ZL.** 2009. Establishing SCID mouse models of B-cell nonHodgkin's lymphoma. Ai Zheng **28:**181–183.
24. **Yoshida M, Rybak RJ, Choi Y, Greenberg SJ, Barcos M, Kawata A, Matsuno F, Tsai H, Seon BK.** 1997. Development of a severe combined immunodeficiency (SCID) mouse model consisting of highly disseminated human B-cell leukemia–lymphoma, cure of the tumors by systemic administration of immunotoxin, and development/application of a clonotype-specific polymerase chain reaction-based assay. Cancer Res **57:**678–685.

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