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Case Study

Supernumerary Incisors in CB6F1 Mice Conditioned with Chemotherapy and Total Body Irradiation before Bone Marrow Transplantation

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Multiple adult female CB6F1 mice presented with supernumerary incisors after preconditioning with chemotherapy and total body irradiation for bone marrow transplantation (BMT). Mice received nonmyeloablative total body irradiation (3 Gy) and either cyclophosphamide or bendamustine, followed by BMT and posttransplantation cyclophosphamide or bendamustine. Here we describe the clinical presentation, μCT findings, and histopathologic evaluation of the affected mice. These analyses confirmed the gross diagnosis and revealed details of the abnormal tooth morphology. We surmise that the combination of total body irradiation and chemotherapy resulted in the abnormal formation of supernumerary incisors. Supernumerary teeth should be considered as a potential confounding factor in tracking weight loss after BMT. These conditions can be managed to allow animals to reach their intended scientific endpoint.

Abbreviations: BMT, bone marrow transplantation; TBI, total body irradiation.

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Bone marrow transplantation (BMT) can be curative for acute and chronic leukemia, lymphoma, aplastic anemia, severe combined immunodeficiency, metabolic disorders, and many other diseases affecting the bone marrow. Successful BMT requires immunosuppression of the recipient before transplantation, known as the conditioning regimen, to facilitate donor cell engraftment as well as after transplantation to reduce the occurrence of graft-versus-host disease.[8,9,11,35] BMT conditioning regimens often have marked side effects. As such, murine models have been used widely in hematopoietic cell transplantation research and have contributed significantly to improvements in the management of patients undergoing BMT.[9,28]

Total body irradiation (TBI) is commonly used during conditioning in both humans and murine models of BMT. The method is fast, technically simple, and results in dose-dependent immunosuppression.[9,21,25] The systemic immunosuppression that results from TBI is nonspecific and causes toxicity to off-target tissues, particularly those with rapidly dividing cell populations. TBI in mice results in radiation sickness, associated with lethargy, weight loss, and diarrhea, with mortality at high doses.[9] Chemotherapy is often used alone or in combination with TBI, and it similarly is associated with nonspecific tissue toxicities. Combining TBI with chemotherapy allows for dose reduction of both agents and decreases the side effects associated with achieving necessary levels of immunosuppression. Cyclophosphamide is the most common agent that is combined with TBI.[6]

Dental abnormalities can occur after TBI as well as chemotherapy in people and have widely been characterized in adult survivors of childhood cancer. These patients have increased risk of dental anomalies including disturbance of mineralization, increased dental caries, crown and root alterations, and delayed or arrested root development.[12-15,18,19,30,35] As such, dental toxicity associated with cancer treatment has been studied in depth in rodent models. Cyclophosphamide reportedly disrupted root development in both young mice[3,18,19,29] and rats.[23,24,27,34,38] In addition, like cyclophosphamide, TBI is associated with dental anomalies in mice, although much less frequently. TBI-associated dental toxicity in C57BL/6 mice exposed to high doses (greater than 10 Gy) included loose, broken, missing, and 'extra' teeth.[25] Other investigators reported overlong, crooked, and broken incisors in NOD/SCID mice after TBI at 3 Gy.[21]

In this report, we present the development of supernumerary incisor teeth in adult CB6F1 mice that received nonmyeloablative TBI (3 Gy, ^{137}Cs) and alkylating chemotherapy before and after TBI. We here present the gross, μCT, and histopathologic analyses of 2 affected mice are provided. To our knowledge, supernumerary (that is, 'extra') teeth have been described to occur only in C57BL/6 mice receiving TBI monotherapy with high doses of radiation (≥14Gy).[25] We suspect that multimodal immunosuppression in these mice may have increased the likelihood and severity of associated dental anomalies. This finding was incidental to the experimental study but represents a possible confounding factor to investigations using this type of immunosuppression. Dental disease should be considered possible comorbidity in murine BMT models.

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Table 1. Treatment group, time (wk) until appearance of dental abnormalities, and summary of gross appearance

Mouse	Group	Treatment (before/after)	Time (wk) after treatment	Gross appearance
1	A	cyclophosphamide/ cyclophosphamide	11	Bilateral buccal mandibular supernumerary incisors
2	A	cyclophosphamide/ cyclophosphamide	11	Bilateral buccal maxillary and mandibular supernumerary incisors
3	A	cyclophosphamide/ cyclophosphamide	14	Bilateral buccal mandibular supernumerary incisors
4	A	cyclophosphamide/ cyclophosphamide	19	Bilateral buccal mandibular supernumerary incisors
5	B	bendamustine/ cyclophosphamide	19	Bilateral buccal mandibular supernumerary incisors

Case Study

Female adult CB6F1 mice from Jackson Laboratory were housed in sterile IVC (Lab Products) in same-sex social groups up to 4 mice per cage. Mice were fed irradiated chow (diet no. 2919, Teklad Global 19% Protein, Envigo, East Millstone, NJ) without restriction, provided reverse-osmosis–filtered water through an automatic watering system (Edstrom), and maintained on a 14:10-h light:dark cycle. These mice were used in a study to improve the outcome and broaden the application of haploidentical BMT by exploring different pre- and posttransplantation treatments using cyclophosphamide (MilliporeSigma, Burlington, MA) and bendamustine (Selleckchem, Houston, TX). The animal use protocol was reviewed and approved by the IACUC and performed in accordance with the *Guide for the Care and Use of Laboratory Animals*[16] and the Public Health Policy.[33] The University of Arizona animal care and use program is AAALAC-accredited.

Cohorts of mice (age, 9 to 11 wk) experienced 4 experimental conditions: group A (n = 8)—cyclophosphamide (225 mg/kg IP) on day –2, TBI on day –1, BMT and spleen cell transplantation on day 0, and cyclophosphamide (150 mg/kg IP) on day 3; group B (n = 8)—bendamustine (50 mg/kg IV) on day –2, with the remaining components as for group A; group C (n = 8)—regimen as for group A except for bendamustine (30 mg/kg IV) on day 3; and group D (n = 8)—as for group B except for bendamustine (30 mg/kg IV) on day 3. At 11 to 19 wk after the experiment, 4 mice from group A and 1 from group B presented with supernumerary incisors. In particular, 5 of the 16 mice given cyclophosphamide after BMT had teeth abnormalities (Table 1), whereas none of the 16 that received posttransplantation bendamustine did. The experiments were performed twice, and supernumerary teeth occurred in both. The irradiator, cyclophosphamide, and bendamustine were used during this same time period in other, unrelated experiments in which treatments yielded expected results. We believe this history rules out the malfunction of the irradiator and variations in drug composition as possible causes of the dental anomalies.

Two affected mice from group A were submitted to pathology for further examination. On gross exam, mouse 1 had bilateral mandibular buccal supernumerary incisors adjacent to and in the same directional orientation as the normal incisors (Figure 1); this mouse was evaluated by µCT also (Figure 2). Mouse 2 had bilateral maxillary and mandibular buccal supernumerary incisors. Histopathology was similar for both mice, yielding a morphologic diagnosis of polydontia with enamel dysplasia of supernumerary incisors (Figure 3). Medial to each normal incisor was a poorly organized supernumerary incisor with a paracentral blood vessel, irregularly oriented and variably sized dental tubules, random lightly basophilic and eosinophilic differential staining of the dentin (variable calcification), and irregular circumferential border (Table 1).

Figure 1. Supernumerary lower incisors in mouse 1.

Discussion

We presented 5 cases of BMT-associated supernumerary teeth in mice, 2 of which were analyzed in detail. Ionizing radiation and alkylating chemotherapy disrupt cell division and target rapidly dividing cell populations, such as hematopoietic and gastrointestinal cells.[4,5,9,10,26] Mouse dental eruption is continuous, with mitotically active cell populations that are inadvertent targets of treatment. Dental progenitor cells (ameloblasts and odontoblasts) are particularly sensitive to toxic effects, whereas the periodontal ligament that is responsible for tooth eruption is resistant.[21,25] Therefore, after toxic insult, teeth still erupt but typically are abnormal (weak, brittle, loose).[21,25] The supernumerary incisors we observed were abnormal and likely developed after progenitor cell damage induced by immunosuppression. Given that 4 of the 5 observed cases occurred in mice that received cyclophosphamide before and after transplantation, whereas the remaining animal received bendamustine before transplantation but cyclophosphamide afterward, it seems likely that cyclophosphamide—and its combination with TBI—makes the greatest contribution to these dental abnormalities.

Of note, our group has extensively used preconditioning with cyclophosphamide or bendamustine and TBI in BALB/c mice, with no supernumerary teeth observed. In addition, we have administered cyclophosphamide or bendamustine to CAF1 mice after BMT without observed effects on teeth.[31] This history indicates that this phenomenon is either strain-specific to C57BL/6 mice or specific to the combination of cyclophosphamide–TBI or bendamustine–TBI conditioning with posttransplantation cyclophosphamide administration.

In the previous report of TBI-associated dental anomalies in C57BL/6 mice, the authors postulated that 'extra teeth' were

Figure 2. µCT image of mouse 1.

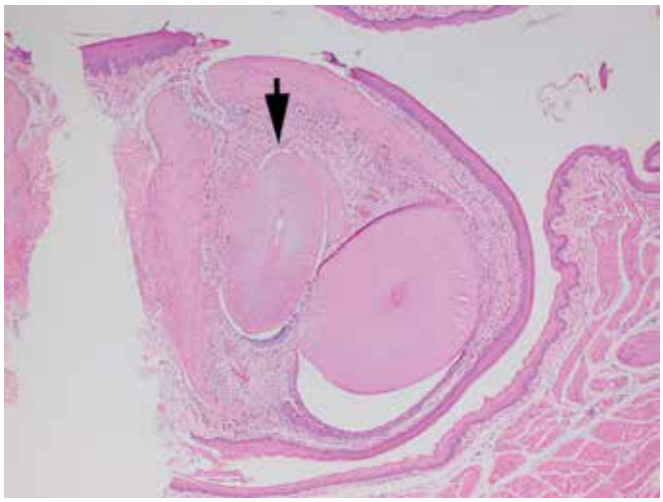

Figure 3. Unilateral cross-section through the mandible showing an abnormal supernumerary tooth (arrow) medial to the normal mandibular incisor. As compared with the normal incisor, the supernumerary incisor has an irregular circumferential border; dental tubules (clear spaces within the incisor) are less frequent and poorly organized; and mineralization (deep basophilic staining) is present. Hematoxylin and eosin stain; magnification, 10×.

a severe abnormality given that it occurred only after high doses of radiation (14 Gy or greater). Abnormalities at lower doses were limited to loose, fractured, and missing incisors.[25] In contrast, mice in the current report developed extra teeth at comparatively low doses of TBI (3 Gy). However, the combination with chemotherapy appears to increase the likelihood and severity of dental toxicity. This additive effect is described in human medicine, where TBI and chemotherapy in combination cause more dental damage than either TBI or chemotherapy alone.[7,12-15,30]

Alternatively, albeit less likely given the lack of effects in other groups, the extra teeth may have been spontaneous and unrelated to experimental manipulation. In rodents, little is known about the incidence or etiology of spontaneous supernumerary, except in mice used specifically for the study of the condition. Most of these mouse models have mutations in the *Wnt* signaling pathway, which is involved in the development of the vestigial dental buds.[1,2,17,20,22,32,36,37] When *Wnt* signal abnormalities exist, vestigial tooth rudiments, which would normally regress, may develop into supernumerary teeth—typically in front of the first premolar (supernumerary diastema teeth).[1,17,36,37] True supernumerary teeth have been reported in mice but are extremely rare.[25]

Animals undergoing BMT require specialized veterinary and husbandry care due to transplantation-related complications.[9] These studies often use weight loss or body condition score as part of the study endpoint criteria. The findings we report here should be remembered as an experimental complication that can be treated effectively. For example, in a previous study, affected NOD/SCID mice presented with weight loss, which resolved when teeth were trimmed and soft food was provided.[21] The same held true in our current study, with mice regaining weight when teeth were trimmed. In particular, care should be taken when using multimodal immunosuppression, because dental toxicity is additive.

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