

A Novel Procedure for Orthotopic Tibia Implantation for Establishment of a More Clinical Osteosarcoma PDOX Mouse Model

NATHANIEL F. WU^{1,2}, JUN YAMAMOTO^{1,3}, MICHAEL BOUVET³ and ROBERT M. HOFFMAN^{1,3}

¹AntiCancer Inc, San Diego, CA, U.S.A.;

²Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA, U.S.A.;

³Department of Surgery, University of California, San Diego, CA, U.S.A.

Abstract. *Background/Aim:* Osteosarcoma is a rare type of malignancy that affects mostly children and adolescents. A new procedure was designed to create an improved patient-derived orthotopic xenograft (PDOX) mouse model of osteosarcoma that more closely mimics osteosarcoma in clinical settings. *Previous osteosarcoma PDOX models involved implanting a tumor fragment near the femur of nude mice in a space created by separating muscle. Materials and Methods:* A hole was created in the tibia of nude mice and an osteosarcoma tumor fragment was implanted directly into the bone. *Results:* This procedure resulted in tumor growth in the bone similar to osteosarcoma tumors found in clinical patients. *Conclusion:* The establishment ratio for this procedure is 80% making it a practical and clinically-relevant model for screening effective therapies for osteosarcoma patients.

Osteosarcoma is a rare type of malignancy that affects mostly children and adolescents. Treatment with a combination of neoadjuvant and adjuvant chemotherapies with surgery improved the prognosis of patients with osteosarcoma (1, 2). However, patients with osteosarcoma often develop chemoresistance, especially patients with metastases, leading to fatality (1-3). Many animal studies using osteosarcoma patient-derived tumors were previously performed to attempt to identify more effective treatments (4-6). However, almost all those studies used a subcutaneous-implantation xenograft

model. Subcutaneous-implantation models are poor surrogate models of cancer in clinical patients and may not reflect effectiveness in treatment studies as accurately as orthotopic-implantation models (7). Tumor implantation at the orthotopic site can lead to metastasis, allowing the tumor to mimic the behavior of tumors observed in patients (8). We previously established an osteosarcoma patient-derived orthotopic xenograft (PDOX) mouse model where the tumor is implanted on the femur. The osteosarcoma PDOX model has identified novel treatment strategies (9-15). The present study demonstrates a new implantation procedure for osteosarcoma to the tibia bone to establish a more clinically-relevant PDOX model of this disease.

Materials and Methods

Mice. This study was conducted on athymic nu/nu nude mice (AntiCancer, Inc., San Diego, CA, USA). The procedures followed an AntiCancer, Inc. Institutional Animal Care and Use Committee (IACUC) protocol specifically approved for this study and in accordance with the principles and procedures outlined in the National Institute of Health Guide for the Care and Use of Animals under Assurance Number A3873-1. All animal procedures have been previously described (9-16).

Patient-derived tumor. The osteosarcoma tumor was previously obtained from a 14-year-old boy with pelvic osteosarcoma as part of a UCLA Institutional Review Board approved protocol (IRB#10-001857). Written informed consent was obtained from the patient (16). The patient was not administered chemotherapy or radiotherapy before the fresh biopsy sample was taken (16).

New surgical orthotopic tibia-implantation procedure to establish a more clinically-relevant osteosarcoma PDOX model. Mice containing subcutaneously-implanted tumors larger than 10 mm in diameter were anesthetized with a ketamine mixture and the tumors were harvested and divided into fragments of 1 mm³. An 8 mm incision was made in either the left or right thigh of nude mice (Figure 1A). The mouse leg was bent at the knee to expose the quadriceps and calf through the incision. The medial tibia was then

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Correspondence to: Robert M. Hoffman, Ph.D., AntiCancer Inc, 7917 Ostrow St, San Diego, CA, 92111, U.S.A. Tel: +1 8586542555, Fax: +1 8582684175, e-mail: all@anticancer.com

Key Words: PDOX, patient-derived orthotopic xenograft, osteosarcoma, tibia implantation.

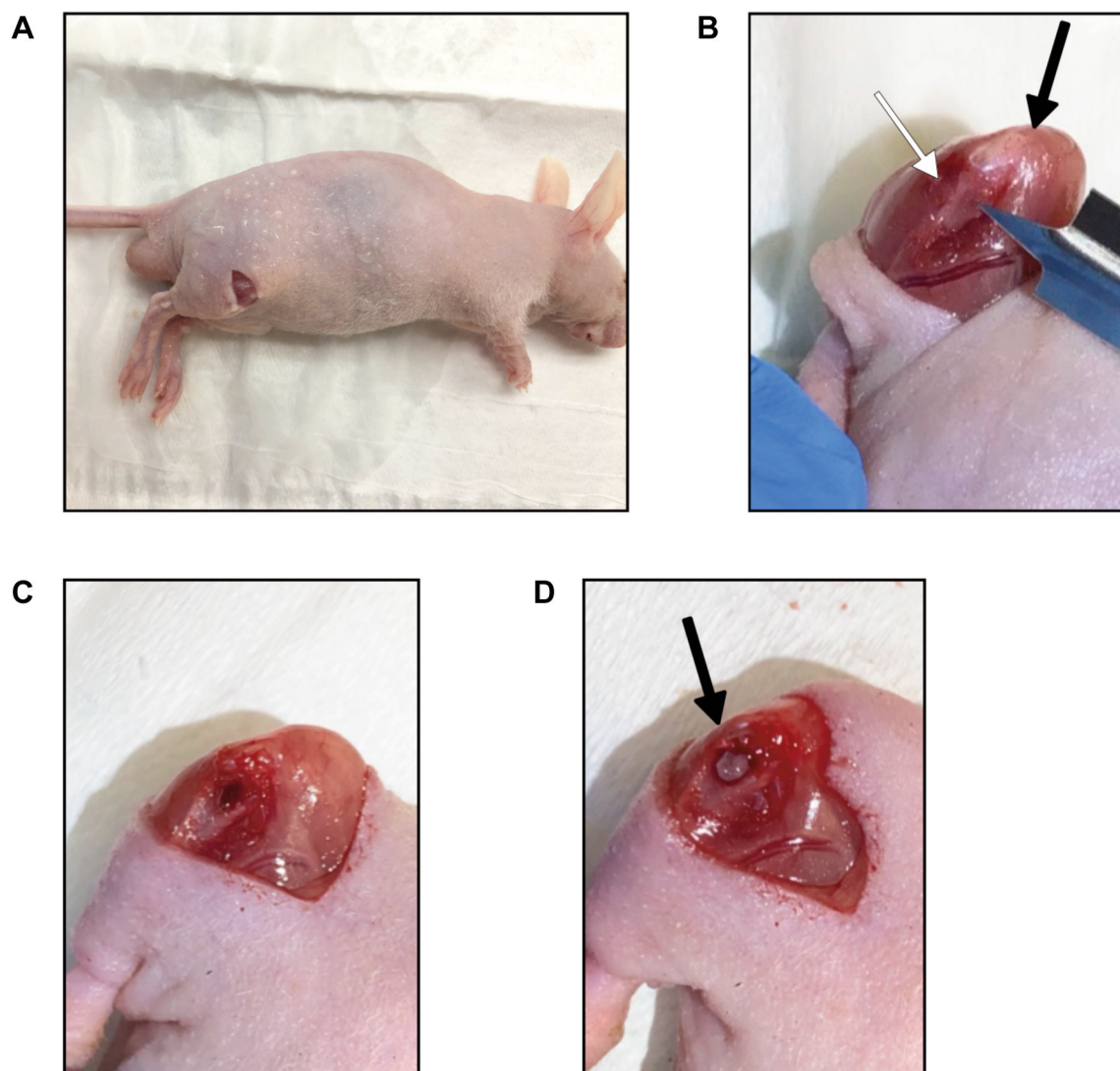


Figure 1. Bone-implantation method for osteosarcoma PDOX. (A) An 8 mm incision made on the skin. (B) Quadriceps and calf exposed by bending the knee. The membrane was dissected away from the tibia bone and muscle. Black arrow indicates the knee cap. White arrow indicates the tibia. (C) After a 5-mm blade was used to puncture a hole and was rotated, a 1 mm diameter hole was formed in the tibia. (D) A 1-mm³ tumor fragment was inserted into the hole. Black arrow indicates the implanted tumor fragment.

visualized. The membrane was dissected to remove any membrane attachments from the lower-extremity muscle (Figure 1B). Then a 5 mm blade was used to puncture the proximal tibia. Once the tibia was punctured and the blade was 2.5 mm into the bone, the blade was rotated with multiple revolutions to create a hole in the bone (Figure 1C). Fine tweezers were used to smoothen and clean the hole. Once a 1 mm diameter hole was visible, a 1 mm³ tumor fragment was inserted into the hole. Fine tweezers were used to push the tumor fragment into the hole so the tumor was not raised above the bone, preventing it from falling out (Figure 1D). The incision was closed with a 5-0 PDS-II suture. Tumor size was measured with calipers 4, 5, and 6 weeks after tumor implantation. Tumor volume was calculated as described (3). All mice were sacrificed 6 weeks after implantation.

Hematoxylin and eosin staining. Fixation, paraffin sectioning, and staining were performed as previously described (10). Hematoxylin and eosin (H&E) staining was performed according to standard protocols (15).

Results

Ten mice were implanted using the new procedure. The tumors grew in 8 mice (80%). All tumors were detectable through the skin (Figure 2A and B). No mice had any gait disorder. The tumor volume significantly increased 4, 5, and 6 weeks after tumor implantation (Figure 3). H&E staining showed that tumors comprised viable, highly-

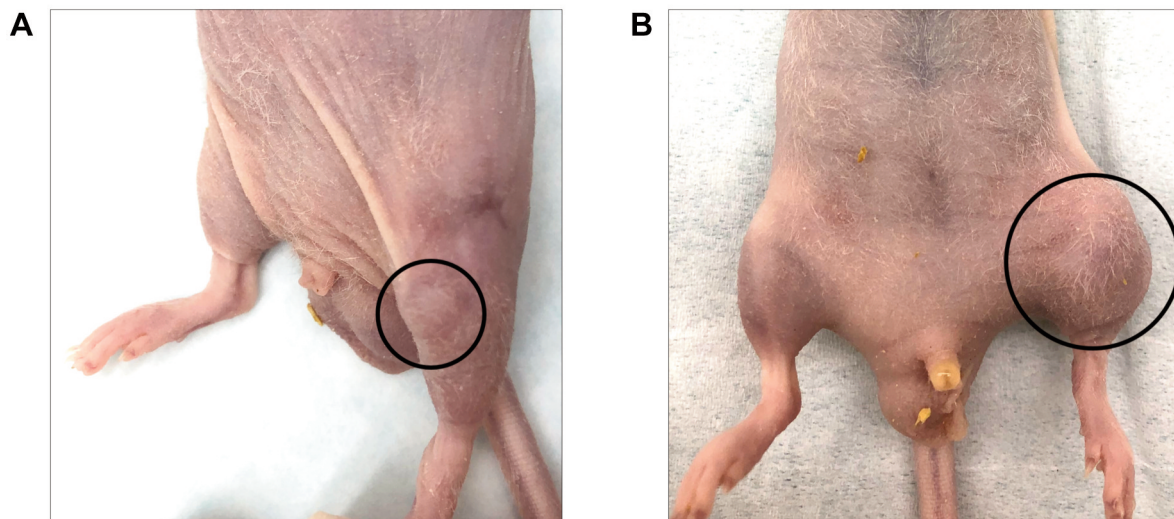


Figure 2. Osteosarcoma PDOX growth in the bone. (A) Twenty-eight days after implantation. (B) Forty-two days after implantation.

dense cancer cells with a pleomorphic spindle shape (Figure 4).

Discussion

In the present study, we established a new procedure of orthotopic implantation of osteosarcoma in the tibia bone. Compared to the previous surgical orthotopic implantation (SOI) osteosarcoma PDOX model, this new procedure is an improvement because it is a more clinically-accurate tumor model, closely mimicking what is observed in osteosarcoma patients.

The primary difference between the new procedure and the previous one is the location of tumor implantation. Instead of placing the tumor in the space between the muscle and bone, the new procedure implants the tumor directly into the bone, more accurately simulating the growth of osteosarcoma tumors in patients. Because the recess in the tibia was smaller than the space in the muscle, the tumor fragments used in these new procedures were 1 mm^3 compared to $3\text{-}4 \text{ mm}^3$ fragments used previously. However, the tumor cells were very aggressive and grew significantly and quickly in the bone despite the relatively small initial tumor size. The establishment ratio of 80% makes this new procedure practical. Moreover, this procedure is versatile. The method of creating a recess in the bone in which a tumor can be implanted can be applied to implantation into any bone. Many more bone-cancer-implantation PDOX models can be designed from this method.

This PDOX mouse model is useful for two reasons. First, individualized clinically-relevant mouse models can be made for patients with osteosarcoma. With a patient tumor,

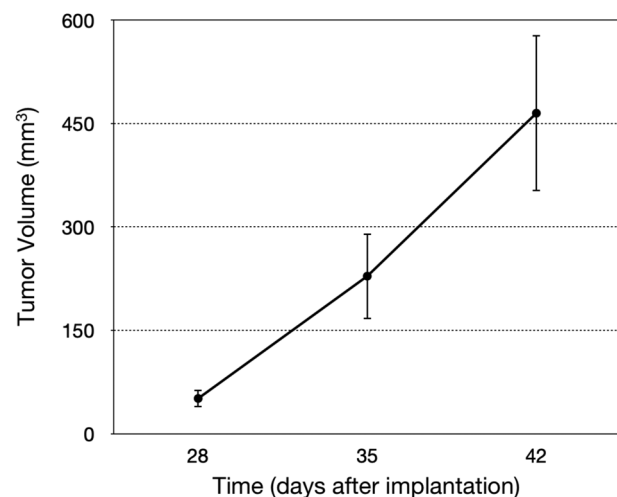


Figure 3. Quantitative osteosarcoma PDOX growth in the bone.

personal mouse models can assist in determining the most effective treatment plan for a certain patient. Second, previous osteosarcoma PDOX models have already identified potentially more effective treatments (9-16). This improved osteosarcoma PDOX model, which closely resembles clinical osteosarcoma, can be used to test novel therapies. The new procedure for tibia osteosarcoma-tumor implantation described in the present report is important, because with these improved clinically-representative mouse models, treatment experiments can be carried out with better translation to patient therapy and better understanding of the biology of the disease, in contrast to subcutaneous sarcoma mouse models (17).

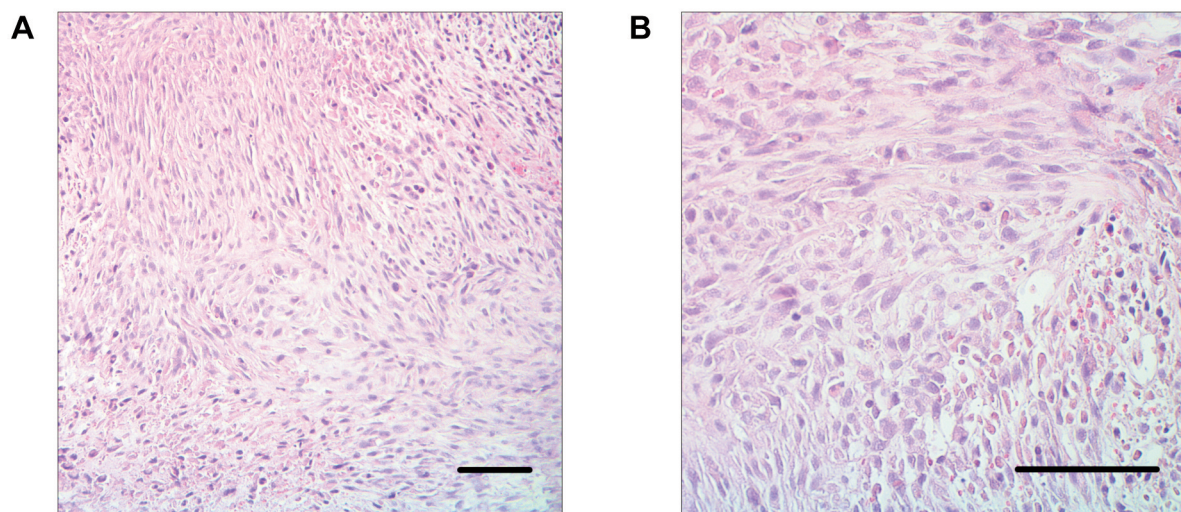


Figure 4. Tumor histology of osteosarcoma PDOX in the bone. Scale bars: 100 μm . (A) 100 \times magnification. (B) 200 \times magnification.

Conflicts of Interest

N.F.W., J.Y., and RMH are or were unsalaried associates of AntiCancer Inc. which performs contract research with PDOX models. The Authors declare that there are no potential conflicts of interest regarding this study.

Authors' Contributions

N.F.W. and J.Y. designed and performed experiments and wrote the paper; R.M.H. gave technical support and conceptual advice. Writing, review, and/or revision of the manuscript: N.F.W., M.B., and R.M.H.

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