Patient-derived Orthotopic Xenograft (PDOX) Nude Mouse Model of Soft-tissue Sarcoma More Closely Mimics the Patient Behavior in Contrast to the Subcutaneous Ectopic Model

YUKIHIKO HIROSHIMA[1,2,3], YONG ZHANG[1], NAN ZHANG[1], FUMINARI UEHARA[1,2], ALI MAAWY[2], TAKASHI MURAKAMI[1,2], SUMIYUKI MII[1,2], MAKO YAMAMOTO[1,2], SHINJI MIWA[1,2], SHUYA YANO[1,2], MASASHI MOMIYAMA[3], RYUTARO MORI[3], RYUSEI MATSUYAMA[3], TAKASHI CHISHIMA[3], KUNIYA TANAKA[3], YASUSHI ICHIKAWA[3], MICHAEL BOUVET[2], ITARU ENDO[3] and ROBERT M. HOFFMAN[1,2]

[1]*AntiCancer, Inc., San Diego, CA, U.S.A.;*
[2]*Department of Surgery, University of California San Diego, San Diego, CA, U.S.A.;*
[3]*Department of Gastroenterological Surgery, Yokohama City University Graduate School of Medicine, Yokohama, Japan*

Abstract. *Aim: Soft-tissue sarcomas are a group of rare mesenchymal carcinomas that include approximately 50 histological types, and account for 1% of all adult cancer cases. The yearly incidence of soft-tissue sarcomas in the USA is approximately 11,280 cases, with an overall mortality of 3,900 deaths per year. Materials and Methods: In this study, we established a patient-derived orthotopic xenograft (PDOX) from a patient with a soft-tissue sarcoma of the retroperitoneum in nude mice and compared it to a subcutaneous patient-derived model of the same tumor for histology. Results: In the PDOX model, a bulky tumor grew in the left retroperitoneum in the same manner as the patient's tumor. Upon histological examination, the majority of the PDOX tissue section comprised sarcomatous high-grade spindle cells of varying sizes, similar to the original patient tumor. In contrast, the majority of the subcutaneously-implanted tumor comprised round to oval cells. Conclusion: These results indicate that the PDOX recapitulated the histology of the original tumor more than the subcutaneous model.*

Soft-tissue sarcomas are a group of rare mesenchymal carcinomas that include approximately 50 histological types, and account for 1% of all adult cancer cases (1). The yearly incidence of soft-tissue sarcomas in the USA is approximately 11,280 cases, with an overall mortality of 3,900 deaths per year (2). The development of new systemic treatments for soft-tissue sarcomas has progressed little in the past few decades. Patients with metastatic soft-tissue sarcomas have a median overall survival of about 12 months. There is a need to find effective therapy for patients with these tumors.

Our laboratory pioneered the patient-derived orthotopic xenograft (PDOX) nude mouse model in the early 1990s (3-8) and demonstrated that the PDOX model, which uses intact tissue for orthotopic transplantation, closely mimics the patient, including the metastatic pattern. In contrast, subcutaneously-implanted patient tumor models, now given such names as "xenopatients" (9), "avatars" (10) and "tumorgrafts" (11), do not mimic the patient since they cannot metastasize. Recent studies from our laboratory have demonstrated that PDOX models can be used to develop fluorescence-guided surgery (12-14), novel therapeutics such as tumor-targeting bacteria (15) and study the tumor microenvironment (TME) (16-18).

In the present study, we established a PDOX nude mouse model with a soft-tissue sarcoma of the retroperitoneum from a patient in nude mice and compared the PDOX to a subcutaneous model for tumor growth and histology.

Materials and Methods

Animals. Male athymic nu/nu nude mice (AntiCancer Inc., San Diego, CA, USA), 4-6 weeks old, were used in this study. Mice were kept in a barrier facility under HEPA filtration. Mice were fed with autoclaved laboratory rodent diet. All mouse surgical procedures and imaging were performed with the animals anesthetized by intramuscular injection of a solution of 50%

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

Key Words: Soft-tissue sarcoma, patient-derived orthotopic xenograft (PDOX), subcutaneous model, nude mouse, histology, spindle cells.

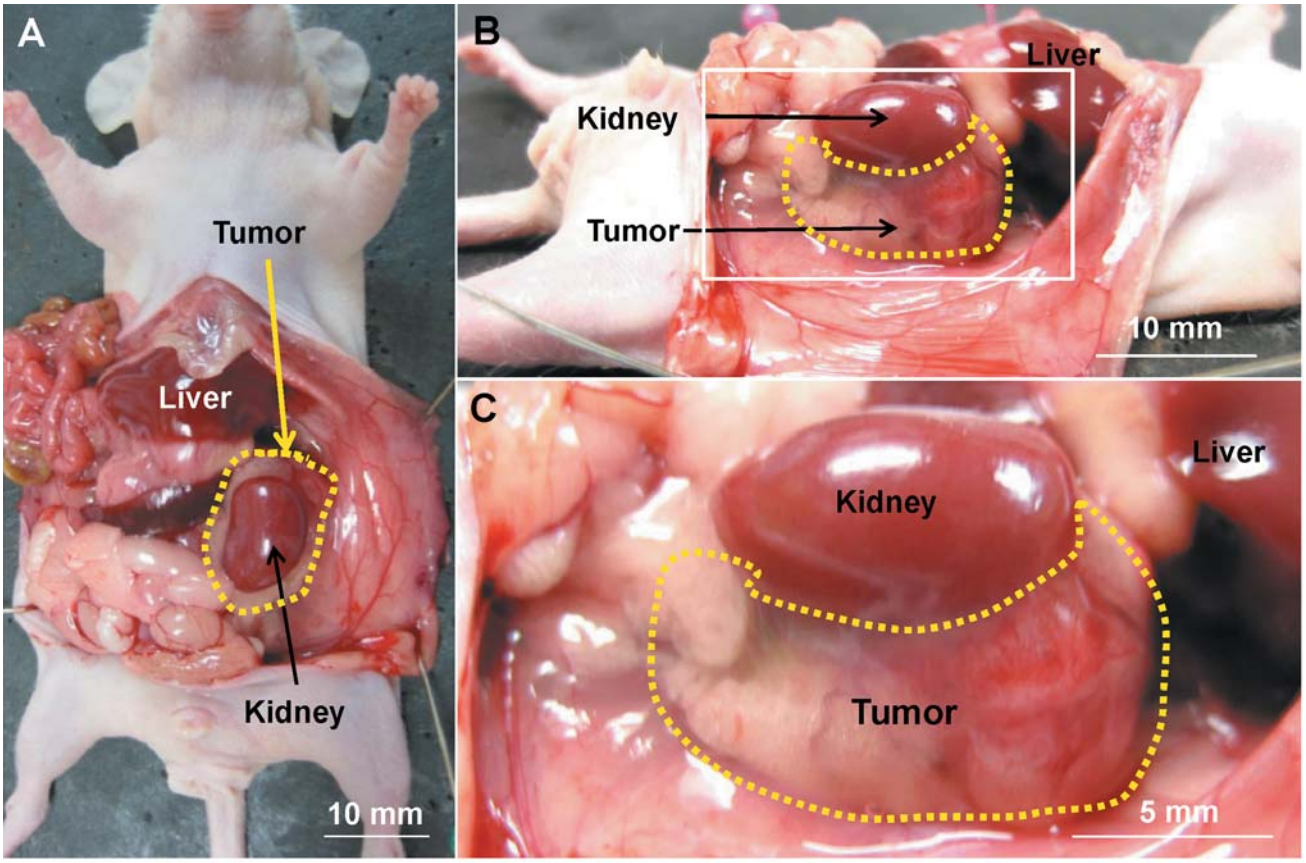

Figure 1. *Features of the patient-derived orthotopic xenograft (PDOX) model of soft-tissue sarcoma growing in nude mice. A: Laparotomy of the soft tissue sarcoma PDOX model. B: Lateral view of the laparotomy of the soft tissue sarcoma PDOX model. C: High magnification image of (B). The areas surrounded by the yellow dashed lines indicate the large primary tumor growing in the retroperitoneal space just behind the left kidney.*

ketamine, 38% xylazine, and 12% acepromazine maleate (0.02 ml). All animal studies were conducted with an AntiCancer Institutional Animal Care and Use Committee (IACUC)-protocol specifically approved for this study and in accordance with the principals and procedures outlined in the National Institutes of Health Guide for the Care and Use of Animals under Assurance Number A3873-1.

Specimen collection. The patient provided written informed consent and samples were procured and the study was conducted under the approval of the Institutional Review Board of the UC San Diego Medical Center.

Establishment of a PDOX of soft-tissue sarcoma. Tumor tissues were obtained from a patient with a soft-tissue sarcoma of the retroperitoneum at biopsy, cut into fragments (3-mm^3) and transplanted to the retroperitoneal space behind the left kidney of nude mice. A small 6- to 10-mm transverse incision was made on the left lateral lumbar region of the mouse through the skin and muscle. Retroperitoneal fat behind the left nude-mouse kidney, corresponding to where the original tumor grew in the patient, was exposed through this incision and split to make space for implantation. A single tumor fragment (3-mm^3) was inserted into this space, which was closed using 8-0 nylon surgical sutures (Ethilon; Ethicon Inc., NJ, USA). On completion, the incision was closed in one layer using 6-0 nylon surgical sutures (Ethilon; Ethicon Inc.). Similar-sized tumor fragments were also transplanted subcutaneously in nude mice using standard techniques.

Tissue histology. Seven weeks after implantation, tumor samples were removed with surrounding normal tissues at the time of resection. Fresh tissue samples were fixed in 10% formalin and embedded in paraffin before sectioning and staining. Tissue sections (5 μm) were deparaffinized in xylene and rehydrated in an ethanol series. Hematoxylin and eosin (H&E) staining was performed according to standard protocols. The sections were examined using a BH-2 microscope (Olympus, Tokyo, Japan) equipped with an INFINITY1 2.0 megapixel CMOS digital camera (Lumenera Corporation, Ottawa, Canada). All images were acquired using INFINITY ANALYZE software (Lumenera Corporation) without post-acquisition processing.

Quantitation of the number of spindle cells in tissue sections. The quantitation of cells was determined by counting cells in four different random fields in the same section at ×200 magnification using the BH-2 microscope (described above). The average number

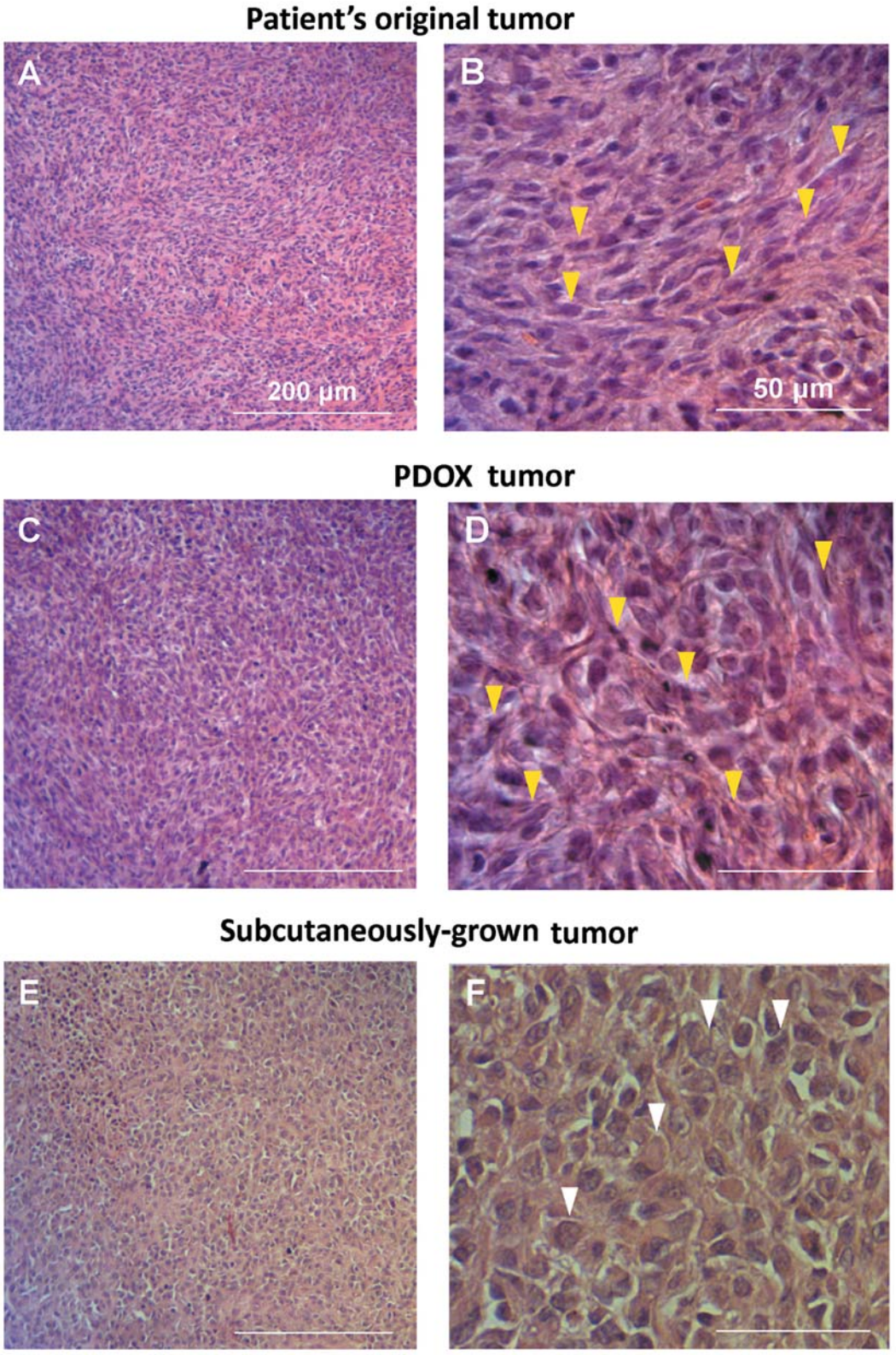

Figure 2. *Histology of original tumor, patient-derived orthotopic xenograft (PDOX) model and subcutaneous-transplant model. Hematoxylin and eosin-stained sections of the patient's original tumor (A, B); PDOX tumor (C, D); and subcutaneously-grown tumor (E, F) at low (A, C, E) and high (B, D, F) magnification. Yellow arrowheads indicate sarcomatous spindle cells. White arrowheads indicate round to oval cells.*

of spindle cells was calculated and data for the original patient tumor, the PDOX tumor and the subcutaneously-grown tumors were compared. Data for each tumor are represented as the mean ± SD.

Statistical analysis. PASW Statistics 18.0 (SPSS, Inc., Quarry Bay, Hong Kong) was used for all statistical analyses. The Student's *t*-test was used to compare continuous variables between two groups. A *p*-value of 0.05 was considered statistically significant for all comparisons.

Results and Discussion

Establishment of a PDOX of soft-tissue sarcoma. Tumor tissues from a patient with a soft-tissue sarcoma of the retroperitoneum were transplanted to the retroperitoneal space behind the left kidney of nude mice. Eight weeks after implantation, a bulky tumor was found growing in the left retroperitoneum in the same manner as the tumor grew in the patient (Figure 1).

PDOX of soft-tissue sarcoma recapitulates the histology of the original tumor. We compared the histology of the PDOX, the subcutaneous transplant and original patient tumors of the soft-tissue sarcoma with H&E staining. The majority of the original patient tumor section was comprised of sarcomatous high-grade spindle cells of varying sizes, demonstrating abundant, finely granular cytoplasm and atypical, pleomorphic, round-to-elongated nuclei with irregular nuclear membranes, an open chromatin pattern and prominent nucleoli (Figure 2A and B). The PDOX tumor had histological structures similar to those of the original tumor (Figure 2C and D). In contrast, the majority of the subcutaneously-grown tumor tissue section was comprised of round to oval cells (Figure 2E and F). The number of spindle cells in the original tumor was 245.3±68.5; in the PDOX tumor was 177.0±11.5; and in the subcutaneous tumor section was 116.0±30.5. The number of spindle cells in the subcutaneous tumor was significantly lower compared to the original tumor and the PDOX tumor (p=0.014 and p=0.01, respectively). The percentage of spindle cells in the original tumor was 62.1±5.3; the PDOX tumor was 49.9±4.5; and the subcutaneous tumor, 28.0±4.5. The percentage of spindle cells in the subcutaneous tumor was significantly lower compared to the original tumor and the PDOX tumor (p<0.001 and p<0.001, respectively), indicating that the PDOX recapitulated the histology of the original tumor, while the subcutaneously grown tumor did not.

In conclusion, the PDOX from the patient with a soft-tissue sarcoma of the retroperitoneum was successfully established in nude mice. This is the first report of a PDOX model of soft-tissue sarcoma. Orthotopic implantation preserved the original histological structure of the patient tumor.

The results of the present report suggest caution with respect to the use of subcutaneously-grown patient tumor mouse models for directing patient therapy (9-11) or drug discovery (19) and suggestions that subcutaneous models are a "breakthrough" (20) when indeed they were developed in 1969 (21). The present results and previous publications (3-8) indicate that orthotopic mouse models are more clinically appropriate.

Acknowledgements

This study was supported in part by National Cancer Institute grant CA183280 and JSPS KAKENHI Grant Numbers 26830081 to Y.H., 26462070 to I.E and 24592009 to K.T.

Dedication

This paper is dedicated to the memory of A. R. Moossa, M.D., and to the memory of James (Barney) Berglund, Jr., who inspired us to find improved therapy of soft-tissue sarcoma.

References

1 Clark MA, Fisher C, Judson I and Thomas JM: Soft-tissue sarcomas in adults. N Engl J Med *353*: 701-711, 2005.

2 Siegel R, Naishadham D and Jemal A: Cancer statistics, 2012. CA Cancer J Clin *62*: 10-29, 2012.

3 Fu X, Guadagni F and Hoffman RM: A metastatic nude-mouse model of human pancreatic cancer constructed orthotopically with histologically intact patient specimens. Proc Natl Acad Sci USA *89*: 5645-5649, 1992.

4 Fu XY, Besterman JM, Monosov A and Hoffman RM: Models of human metastatic colon cancer in nude mice orthotopically constructed by using histologically intact patient specimens. Proc Natl Acad Sci USA *88*: 9345-9349, 1991.

5 Fu X and Hoffman RM: Human ovarian carcinoma metastatic models constructed in nude mice by orthotopic transplantation of histologically-intact patient specimens. Anticancer Res *13*: 283-286, 1993.

6 Fu X, Le P, and Hoffman RM. A metastatic-orthotopic transplant nude-mouse model of human patient breast cancer. Anticancer Res *13*: 901-904, 1993.

7 Furukawa T, Fu X, Kubota T, Watanabe M, Kitajima M and Hoffman RM: Nude mouse metastatic models of human stomach cancer constructed using orthotopic implantation of histologically intact tissue. Cancer Res *53*: 1204-1208, 1993.

8 Wang X, Fu X and Hoffman RM: A new patient-like metastatic model of human lung cancer constructed orthotopically with intact tissue *via* thoracotomy in immunodeficient mice. Int J Cancer *51*: 992-995, 1992.

9 Bertotti A, Migliardi G, Galimi F, Sassi F, Torti D, Isella C, Corà D, Di Nicolantonio F, Buscarino M, Petti C, Ribero D, Russolillo N, Muratore A, Massucco P, Pisacane A, Molinaro L, Valtorta E, Sartore-Bianchi A, Risio M, Capussotti L, Gambacorta M, Siena S, Medico E, Sapino A,Marsoni S, Comoglio PM, Bardelli A and Trusolino L: A molecularly annotated platform of patient-derived xenografts ("xenopatients") identifies HER2 as an effective therapeutic target in cetuximab-resistant colorectal cancer. Cancer discovery *1*: 508-523, 2011.

10 Dong X, Guan J, English JC, Flint J, Yee J, Evans K, Murray N, Macaulay C, Ng RT, Gout PW, Lam WL, Laskin J, Ling V, Lam S and Wang Y: Patient-derived first generation xenografts of non-small cell lung cancers: promising tools for predicting drug responses for personalized chemotherapy. Clin Cancer Res *16*: 1442-1451, 2010.

11 DeRose YS, Wang G, Lin YC, Bernard PS, Buys SS, Ebbert MT, Factor R, Matsen C, Milash BA, Nelson E, Neumayer L, Randall RL, Stijleman IJ, Welm BE and Welm AL: Tumor grafts derived from women with breast cancer authentically reflect tumor pathology, growth, metastasis and disease outcomes. Nat Med *17*: 1514-1520, 2011.

12 Hiroshima Y, Maawy A, Metildi CA, Zhang Y, Uehara F, Miwa S, Yano S, Sato S, Murakami T, Momiyama M, Chishima T, Tanaka K, Bouvet M, Endo I and Hoffman RM: Successful fluorescence-guided surgery on human colon cancer patient-derived orthotopic xenograft mouse models using a fluorophore-conjugated anti-CEA antibody and a portable imaging system. J Laparoendosc Adv Surg Tech A *24*: 241-247, 2014.

13 Hiroshima Y, Maawy A, Sato S, Sato S, Murakami T, Uehara F, Miwa S, Yano S, Momiyama M, Chishima T, Tanaka K, Bouvet M, Endo I and Hoffman RM: Hand-held high-resolution fluorescence imaging system for fluorescence-guided surgery of patient and cell-line pancreatic tumors growing orthotopically in nude mice. J Surg Res *187*: 510-517, 2014.

14 Hiroshima Y, Maawy A, Zhang Y, Murakami T, Momiyama M, Mori R, Matsuyama R, Katz MH, Fleming, JB, Chishima T, Tanaka K, Ichikawa, Y, Endo I, Hoffman RM, and Bouvet M. Metastatic recurrence in a pancreatic cancer patient derived orthotopic xenograft (PDOX) nude mouse model is inhibited by neoadjuvant chemotherapy in combination with fluorescence-guided surgery with an anti-CA 19-9-conjugated fluorophore. PLoS One *9*: e114310, 2014.

15 Hiroshima Y, Zhao M, Maawy A, Zhang Y, Katz MH, Fleming JB, Uehara F, Miwa S, Yano S, Momiyama M, Suetsugu A, Chishima T, Tanaka K, Bouvet M, Endo I and Hoffman RM: Efficacy of Salmonella typhimurium A1-R versus chemotherapy on a pancreatic cancer patient-derived orthotopic xenograft (PDOX). J Cell Biochem *115*: 1254-1261, 2014.

16 Suetsugu A, Katz M, Fleming J, Truty M, Thomas R, Saji S, Moriwaki H, Bouvet M and Hoffman RM: Non-invasive fluorescent-protein imaging of orthotopic pancreatic-cancer-patient tumorgraft progression in nude mice. Anticancer Res *32*: 3063-3068, 2012.

17 Suetsugu A, Katz M, Fleming J, Truty M, Thomas R, Saji S, Moriwaki H, Bouvet M, and Hoffman RM. Imageable fluorescent metastasis resulting in transgenic GFP mice orthotopically implanted with human-patient primary pancreatic cancer specimens. Anticancer Res *32*: 1175-1180, 2012.

18 Suetsugu A, Katz M, Fleming J, Truty M, Thomas R, Saji S, Moriwaki H, Bouvet M, and Hoffman RM. Non-invasive fluorescent-protein imaging of orthotopic pancreatic-cancer-patient tumorgraft progression in nude mice. Anticancer Res *32*: 3063-3068, 2012.

19 Tentler JJ, Tan AC, Weekes CD, Jimeno A, Leong S, Pitts TM, Arcaroli JJ, Messersmith WA and Eckhardt SG: Patient-derived tumour xenografts as models for oncology drug development. Nat Rev Clin Oncol *9*: 338-350, 2012.

20 Couzin-Frankel J. The littlest patient. Science *346*: 25-27, 2014.

21 Rygaard J, Poulsen CO. Heterotransplantation of a human malignant tumour to "nude" mice. Acta Path Microbiol Scand *77*: 758-760, 1969.

Received October 14, 2014
Revised November 4, 2014
Accepted November 5, 2014