Development and characterization of a patient-derived orthotopic xenograft of therapy-resistant breast cancer

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Abstract. Numerous years of cell line-based studies have enhanced the current understanding of cancer and its treatment. However, limited success has been achieved in treating hormone receptor-positive, HER2-negative metastatic breast cancers that are refractory to treatment. The majority of cancer cell lines are unsuitable for use as pre-clinical models that mimic this critical and often fatal clinical type, since they are derived from treatment-naive or non-metastatic breast cancer cases. The aim of the present study was to develop and characterize patient-derived orthotopic xenografts (PDOXs) from patients with endocrine hormone receptor-positive, HER2-negative metastatic breast cancer who had relapsed on therapy. A patient who progressed on endocrine hormone therapy provided her tumor via a biobank. This tumor was implanted in mice. It was then serially passaged by implanting PDOX tumor fragments into another set of mice to develop further generations of PDOXs. These tissues were characterized using various histological and biochemical techniques. Histological, immunofluorescence and western blot analyses indicated that the PDOX tumors retained a similar morphology, histology and subtype-specific molecular features to that of the patient's tumor. The present study successfully established PDOXs of hormone-resistant breast cancer and characterized them in comparison with those derived from the original breast cancer tissue of the patient. The data highlight the reliability and usefulness of PDOX models for studies of biomarker discovery and preclinical drug screening. The present study was registered with the clinical trial registry of India (CTRI; registration no. CTRI/2017/11/010553; registered on 17/11/2017).

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Introduction

Breast cancer accounts for the highest mortality among female cancer patients in India (1). It is also the most prevalent cancer type among Indian females with an estimated age-adjusted rate of 25.8 per 100,000 women and a mortality rate of 12.7 per 100,000 women (2). India has a worse survival outcome for patients with breast cancer compared with western countries (3). Specifically, 90,408 mortalities are reported in India due to breast cancer as per the Globocan data 2020 (3). Advances in novel therapies remain untranslated to improved outcomes for patients with therapy-resistant cancer, which remains incurable (4).

An existing challenge in pre-clinical research and drug discovery is the lack of accurate *in vitro* and *in vivo* models that mimic the patients' phenotypes of resistance. Immortalized cell lines and xenograft models derived from such cell lines are markedly different from patients' tumors in terms of molecular and genetic profiles (5). Traditional cancer cell lines are usually grown clonally in plastic flasks with uniform morphology of undifferentiated phenotypes. These cell line

models acquire irreversible genetic and behavioral changes upon serial passaging (6,7). Such pre-clinical models also lack stromal elements that are unable to mirror the architecture and heterogeneity of human tumors (7-9). Guo *et al* (10) have reported poor cancer type specificity in cancer cell lines, suggesting that they deviate both histologically, pathologically and molecularly from patient tumors. Their data demonstrate a high degree of similarity for molecular profiles of human tumors with subsequently established patient-derived xenografts (PDXs) from these tumors as compared to cell line-based xenografts or cell lines (10).

PDXs, also termed patient avatars, retain several of the molecular and functional features of patients' native tumors. PDXs are reliable models for investigating tumor heterogeneity and identifying drug targets for personalized medicine. Over the years, several groups have developed and reported the molecular characterization of PDX models for preclinical drug screening programs worldwide (11-13). A recent study has reported that a novel claudin-low triple-negative breast cancer PDX model maintains a histopathological phenotype throughout subsequent passages in mice, which holds promise for preclinical drug testing (12). Ramani *et al* (13) have highlighted the usage of PDX models for assessing the impact and outcome of different anticancer therapeutics on circulating tumor cell shedding and metastasis in breast cancer. However, a limited number of preclinical PDX models have been established from hormone receptor-positive, HER2/Neu-negative patients with breast cancer who have progressed on endocrine hormone therapy.

The present study aimed to establish a preclinical patient-derived orthotopic xenograft (PDOX) model from a breast cancer patient with the aforementioned phenotype for preclinical drug screening. The development and serial passage of this PDOX model were successful. In addition, multiple generations of PDOX-derived breast tumor tissues were compared along with the patient's tissues, both histopathologically and at the molecular level. The expression levels of specific biomarkers for luminal, epithelial and mesenchymal phenotypes were analyzed by immunofluorescence and western blot analyses. The data indicated that the newly developed PDOX was a suitable model system for preclinical drug screening, biomarker development and personalized treatment for this hormone therapy-resistant patient.

Materials and methods

Patient recruitment and biobanking. A hormone receptor-positive, HER2/Neu-negative patient who progressed on endocrine hormone therapy participated in the present study. The study was approved by Institutional Ethics Committee III of the Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), and registered with the clinical trial registry of India (CTRI; registration no. CTRI/2017/11/010553; registered on 17/11/2017). The patient's sample was deposited in a biobank at the time of biopsy in a magnetic-activated cell sorting tissue storage solution (Miltenyi Biotec, Inc.). Half of the tissues were used for the development of PDOX and the other half was used for the establishment of primary cultures from patients' tumors. These dissected tissues were fixed in formalin solution.

Development of PDOX model and in vivo passaging. The animal procedures were approved by and performed in compliance with the guidelines of the Institutional Animal Care and Use Committee of the National Centre for Cell Science (Pune, India). The breast tumor tissues were minced, collected in 1X Dulbecco's PBS and centrifuged at 123 x g for 10 min at room temperature. Non-dissociated tissue fragments (5-10 pieces of <1 mm in size) were administered orthotopically along with 1:1 Matrigel® (Corning, Inc.) into the right inguinal mammary fat pad of a single female NOD/SCID mouse (age, 8 weeks; body weight, 19 g), which was obtained from the institutional animal facility, National Centre for Cell Science (Pune, India). The mouse was maintained at the in-house pathogen-free facility with *ad libitum* access to sterile food and chlorinated sterile water at 22±1˚C temperature with 55±2% humidity and a 12-h light/dark cycle, and the tumor gradually developed. Following the generation of palpable tumors (approximately within 180 days from date of implantation), the tumor volume was measured using a digital caliper twice a week. The mice were sacrificed when the tumors attained a volume of >2,000 mm^3 and a diameter of >20 mm. The mice were sacrificed using CO_2 asphyxiation by maintaining the CO_2 flow rate at 30-70% of the cage volume per minute to minimize sudden distress and pain. Death of the mice was verified by observing the cessation of breath and heartbeat, as well as areflexia as per the guidelines of the Institutional Animal Care and Use Committee of the National Centre for Cell Science, India. The tumors were excised and the weights were measured. A part of the tumor tissue was again implanted orthotopically into the right mammary fat pad of the next set of female NOD/SCID mice (n=1-3) for the development of subsequent generations of PDOXs.

Hematoxylin and eosin (H&E) staining. Formalin-fixed and paraffin-embedded tissue sections with 5-μm thickness were stained with H&E. Patients' and PDOX tumor sections were deparaffinized using 3 rounds of fixation in xylene and rehydration in descending grades of ethanol (100, 95 and 75%), followed by distilled water at room temperature. The tissue sections were incubated with a hematoxylin solution for 8 min and washed with water. The sections were soaked in 70% ethanol solution containing 1% HCl and washed again. Subsequently, they were stained with eosin for 5 min and rinsed with absolute alcohol and xylene for 5 min each at room temperature. The images were captured using a bright-field microscope and analyzed by an expert histopathologist.

Western blot analysis. The tumor tissues from various generations were lysed using RIPA lysis buffer. Following centrifugation, the cleared supernatant was used to measure the total proteins using Bradford's method. The lysates containing an equal amount of total protein (30 μg) were resolved by 10 and 12.5% SDS-PAGE. Protein was transferred onto PVDF membrane (Bio-Rad Laboratories, Inc.) and further analyzed by western blot analysis. The expression levels of ERα, PR, HER2, E-cadherin, N-cadherin and vimentin in tumor tissues derived from different generations of PDOX models were analyzed using their respective primary antibodies, namely anti-ERα (cat. no. ab32063; 1:1,000 dilution; Abcam), anti-PR (cat. no. 8757; 1:1,000 dilution; Cell Signaling Technology,

Inc.), anti-HER2 (cat. no. ab2428; 1:1,000 dilution; Abcam), anti-E-cadherin (cat. no. ab1416; 1:1,000 dilution; Abcam), anti-N-cadherin (cat. no. ab18203; 1:1,000 dilution; Abcam) and anti-vimentin (cat. no. sc-7558; Santa Cruz Biotechnology, Inc.; 1:1,000 dilution), followed by incubation for 1 h at room temperature with specific secondary antibodies, including goat anti-rabbit IgG HRP (cat no. 114038001A; Genei Laboratories Pvt. Ltd.), rabbit anti-mouse IgG HRP (cat no. 114058001A; Genei Laboratories Pvt. Ltd.) and rabbit anti-goat IgG HRP (cat no. 114048001A; Genei Laboratories Pvt. Ltd.) at 1:2,000 dilution. All the blots were visualized using Clarity Western ECL (Bio-Rad Laboratories, Inc.) reagent.

Establishment of primary culture. The primary cultures were established as previously described with minor modifications (14). The clinical specimens were aseptically transferred into DMEM (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 4% penicillin and streptomycin solution. The tissue specimens were washed twice with 1X PBS containing an antibiotic solution. The tissue specimens were cut into fine pieces and incubated in 0.15% collagenase II and collagenase IV (Gibco; Thermo Fisher Scientific, Inc.) solution in a water bath at 37˚C for 2 h for proper enzymatic digestion. Following incubation, the tissue specimens were centrifuged at 123 x g for 10 min at room temperature. The pellets were resuspended in DMEM supplemented with 20% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.), 25 μl hydrocortisone (Calbiochem), 10 μl insulin (Gibco; Thermo Fisher Scientific, Inc.), 5 μl epidermal growth factor (Peprotech) and a solution containing 2% penicillin and streptomycin (Gibco; Thermo Fisher Scientific, Inc.). The cells were grown in a 95% humidified incubator with 5% CO_2 at 37˚C. When the cultures were established, the amount of FBS was reduced to 10% to maintain the selective growth of cancer epithelial cells.

Characterization of primary cultures. Primary cultures were characterized by immunofluorescence as previously described (15). The primary culture cells were grown on coverslips. When the cells were confluent, they were fixed in 2% paraformaldehyde for 20 min, quenched with 100 mM glycine for 10 min, permeabilized with 0.1% Triton-X100 for 10 min and incubated with 10% FBS in PBS to block non-specific binding for 1 h at room temperature. The cells were incubated overnight at 4˚C with primary antibodies to the following proteins: estrogen receptor (ER)-α (cat. no. ab32063; Abcam), progesterone receptor (PR) (cat. no. ab8757; Cell Signaling Technology, Inc.), HER2 (cat. no. ab2428; Abcam), E-cadherin (cat. no. sc-7870; Santa Cruz Biotechnology, Inc.), N-cadherin (ab18203; Abcam), vimentin (sc-7558; Santa Cruz Biotechnology, Inc.), Ki-67 (sc-23900; Santa Cruz Biotechnology, Inc.), α-smooth muscle actin (SMA; ab5694; Abcam) and pan-cytokeratin (cat. no. C5992; Sigma-Aldrich; Merck Millipore) at 1:100 dilution. Fluorescent-labeled secondary antibodies such as goat anti-mouse (cat. no. AP124C), donkey anti-rabbit (cat. no. AP182C) and donkey anti-goat (cat. no. AP180C) Cy3 antibodies were added to cells at 1:200 dilution and incubated for 1 h at room temperature. The cells were visualized using a confocal microscope (Leica Microsystems).

Statistical analysis. The data were analyzed and graphs were prepared using SigmaPlot version 10.0 (Systat software).

Results

Patient and clinical phenotype. A 51-year-old post-menopausal female with no comorbidities was referred to the Tata Memorial Hospital (Mumbai, India) in October 2013 with a 5-cm lump in the left breast and a 2-cm lymph node (left axilla). The case had been determined to be positive for the presence of a tumor based on a computerized tomography scan. The patient had previously received four cycles of cyclophosphamide, adriamycin and 5-fluorouracil prior to registering at our center. A bone scan revealed the presence of asymptomatic oligometastatic disease with suspected osteoblastic lesions in the ribs. The patient provided consent for further clinical treatment options and agreed to the treatment with radical curative intent by simple mastectomy. Subsequently, the patient received localized radiation therapy (RT) at the site of excision, taxane-based chemotherapy and aromatase inhibitors, which were completed in April 2014. The patient was disease-free for the next two years and presented with a recurring disease in the liver in October 2016. Therapy with tamoxifen and paclitaxel was initiated, along with zoledronic acid. The patients also received palliative RT to the D4-D6 region to alleviate symptomatic pain. The patient remained stable on tamoxifen in the absence of disease progression for 1 year. In 2017, following the appearance of new metastatic nodules in the liver, the patient received therapy containing exemestane. The patient remained stable on exemestane for another year prior to the appearance of new nodules in the lung, which were consistent with the presence of lung metastatic disease. The patient provided consent to being recruited for the present study in 2018. A biopsy was performed in 2-3 tumor cores from the patient's liver using ultrasound guidance. The tissues were added to a biobank for subsequent processing. The patient was subsequently treated with capecitabine therapy along with concurrent palliative RT. The patient progressed within two months and received therapy based on a protocol of gemcitabine and carboplatin. The patient responded to therapy for six months and had stable disease. Subsequently, disease progression was observed in early 2019. The patient received letrozole and zoledronic acid followed by palliative RT. The patient continued receiving therapy containing letrozole and zoledronic acid in 2019, exhibited stable disease and was alive at the last follow-up in April 2022.

Histopathological analysis of the patient's tumor. The histopathological examination of the tumor resected in 2013 identified it as an infiltrating duct carcinoma of the cribriform type with a high nuclear grade (grade III) and necrosis. The tumor exhibited a focal micropapillary pattern and extracellular mucin with a modified Bloom Richardson Score of 3+3+2=8 in the absence of Paget's disease (Fig. 1A). The tumor exhibited positive staining for ER (all-red score, 8) and PR (all-red score, 8), as determined by immunohistochemistry (IHC) (Fig. 1A-C) and negative for HER2 expression on the Ventana system (score +1; data not shown). Histopathological assessment of lymph nodes assessed by axillary clearance

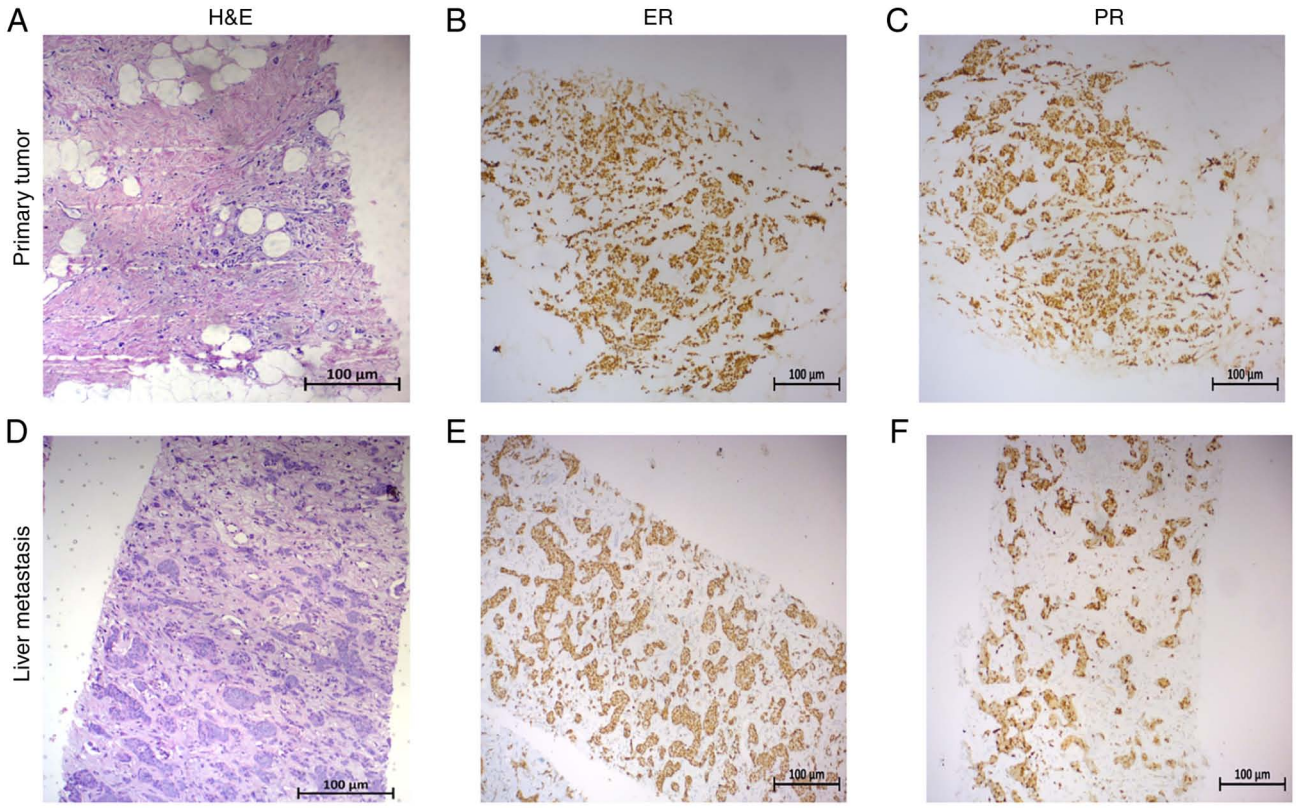

Figure 1. Histochemical analysis of patient's primary tumor and liver metastasis. (A) H&E staining of primary breast tumor after surgical resection indicates infiltrating ductal carcinoma of the cribriform type with high-grade nuclei and necrosis. (B) ER staining revealed that 100% of tumor cells exhibited strong staining with an all-red score of 8/8. (C) PR staining revealed that 100% of tumor cells exhibited strong staining with an all-red score of 8/8. (D) H&E staining of biopsied liver metastasis tumor indicates consistency in histological pattern with resected primary tumor. No native liver parenchyma is observed in this tissue. (E) ER staining revealed that 100% of biopsied tumor cells from the liver exhibited strong staining with an all-red score of 8/8. (F) PR staining revealed that 100% of biopsied tumor cells from the liver exhibited strong staining with an all-red score of 8/8 (scale bars, 100 μm; magnification, x100). ER, estrogen receptor; PR, progesterone receptor.

during surgery revealed the presence of cancer cells in 10/20 lymph nodes assessed. Histopathological re-examination of the tumor biopsied from the patient's liver at progression in January 2018 revealed consistency with the resected tumor prior to five years. The tumor also demonstrated positive staining for ER (all-red score, 8) and PR (all-red score, 8; Fig. 1D-F) and negative for HER2 expression based on the Ventana system (score +1; data not shown).

Generation and serial passaging of breast cancer PDOX. Subcutaneous tumor PDX models are standard pre-clinical models that are widely used to study treatment response. However, they also exhibit low clinical relevance and biological features to patients' tumors as compared to orthotopic PDXs. Furthermore, other major limitations of the subcutaneous models include a lower potential to metastasize and failure to accurately mimic the tumor microenvironment and imitate a patient's clinical treatment response. In contrast to these observations, PDOXs may accurately and precisely epitomize the clinical behavior and treatment response of a patient's unique cancer (11).

In the present study, a breast PDOX model was developed using hormone-resistant-breast cancer by orthotopically implanting tumor fragments into the mammary fat pad of female NOD/SCID mice. The tissues were mixed with Matrigel to mimic the organ microenvironment prior to implantation. The rate of successful engraftment from the patient tumors to the NOD/SCID mice was estimated to be 20% (3 PDOXs were established out of 15 samples implanted; data not shown), which was consistent with previously reported studies (16,17). However, the *in vivo* passaging rate was considerably higher (~90%) following the establishment of PDOXs. Following 70 days of implantation, the tumor was grown to a palpable size (Generation 1; G1). The tumor was grown to the endpoint volume, (~2,000 mm^3) within 90 days after the observation of the palpable tumors. Serial passaging of established PDOXs was performed in the next cohort of immunocompromised mice to establish G2 PDOXs. PDOXs exhibited an unstable growth pattern. The time required to attain palpable tumors (tumor take) for G2 was 60 days, whereas that for G3 was ~40 days. The time required to reach the tumor burden from the stage of palpable tumors for G2 was 60 days, while that for G3 (to reach the same volume as G1) was 50 days (Fig. 2A and B). The time periods required to develop palpable tumors and to reach the tumor burden for G4 were considerably decreased compared with those of the G1-G3 generations of PDOXs. This is likely due to the corresponding patient having undergone chemotherapy prior to surgery. Therefore, PDOXs exhibited signs of chemotherapy-induced differentiation and slow tumor-cell proliferation in the lower passage and aggressive growth in advanced cohorts (Fig. 2A and B).

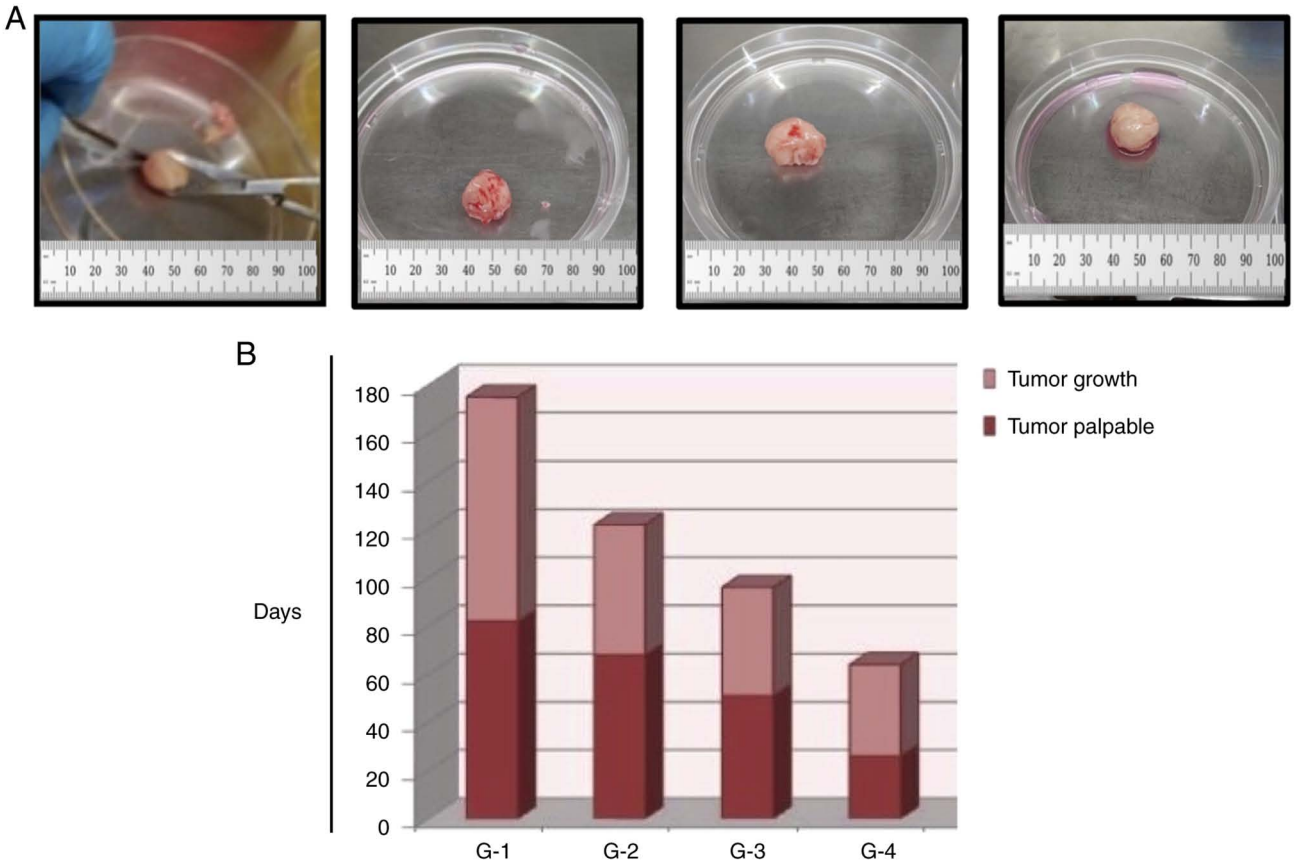

Figure 2. Generation of PDOXs from hormone therapy-resistant breast cancer. The patient's tumor fragments were implanted to develop PDOXs and serially passaged. Tumors were excised after reaching a certain volume that was monitored with digital calipers. (A) Photographs of resected G1-G4 PDOXs tumors (scale in cm). (B) Bar graph representing time to tumor palpability and tumor burden of G1-G4 PDOXs tumors. PDOX, patient-derived orthotopic xenograft.

PDOXs reflect the histological traits of primary tumors. The tissue sections derived from primary tumors and PDOXs were stained with H&E to assess their histological features and determine growth stability and tissue morphology. The histological reports of primary tumors and PDOXs revealed similar grade histology represented by minimal to maximal nuclear polymorphism and vessel formation, numerous mitotic activities with occasional abnormal mitoses including atypical mitotic figures and areas of necrosis (Figs. 1A and 3A-D, Table I). Mitotic activity and necrosis were significantly increased from G1 to G4 (Fig. 3A-D, Table I). The increasing trend was in parallel with the unstable growth patterns observed in the PDOXs (Fig. 2B, Table I). The tumor tissues of different passages of PDOXs were subjected to immunofluorescence and western blot analyses to further characterize the expression of subtype- and epithelial-to-mesenchymal transition (EMT)-specific markers. The results indicated that the expression levels of breast cancer-specific markers, such as ERα and PR, as well as those of the EMT markers, such as E-cadherin, N-cadherin and vimentin, were similar in the different passages of PDOXs (Fig. 4A-C). To further characterize their subtype, the primary cultures were established and immunofluorescence analysis was performed to examine the expression levels of ERα, PR and HER2, and those of the epithelial- and mesenchymal-specific markers, such as E-cadherin, N-cadherin, vimentin and α-SMA. The expression levels of subtype-specific markers, such as ERα, PR and Ki-67, were observed in the primary culture derived from G1 PDOXs and were mostly retained in primary culture of G4 PDOXs. The epithelial and mesenchymal markers, such as E-cadherin, N-cadherin, vimentin and pan-cytokeratin, were also similar between primary cultures of G1 and G4 PDOXs (Fig. 5A and B).

From these observations, it was suggested that the histological and molecular features of PDOXs were mostly retained in different passages of PDOXs. All these data demonstrated the successful development of the hormone therapy-resistant breast cancer PDOX models from an Indian patient. These models were extended to four generations and their luminol-positive features were characterized compared with those of the patient's tissues. These models will be used for drug screening and the development of therapeutically relevant biomarkers using proteomic and genomic-based approaches. All of these works are in progress and will be published soon in a separate article.

Discussion

Patients who progress following multiple rounds of therapy constitute a major proportion of patients with cancer who attain mortality. Such cancers are difficult to treat and lead to rapid degeneration of patient health (18-20). In the majority of these cases, all potential therapeutic interventions are exhausted in the treatment of these patients. Therefore, the

Table I. Histopathological analysis of patient-derived orthotopic xenografts.

Generation of P014R	Cell infiltration in tumor	Angiogenesis	Mitotic activity/hpf	Abnormal mitosis	Tumor giant cells	Nuclear polymorphism	Tumor necrosis
1	L+; M focal	Focal	1-2	Occasional	Absent	++	++
2	L ++; M++	Focal	2-3	Occasional	Absent	++	++
3	L++; N+	+	5-6	Occasional	Absent	+++	+++
4	L+; N++	++	8-10	Occasional	Absent	+++	++++

L, lymphocytes; M, macrophages; N, neutrophils; hpf, high-power field.

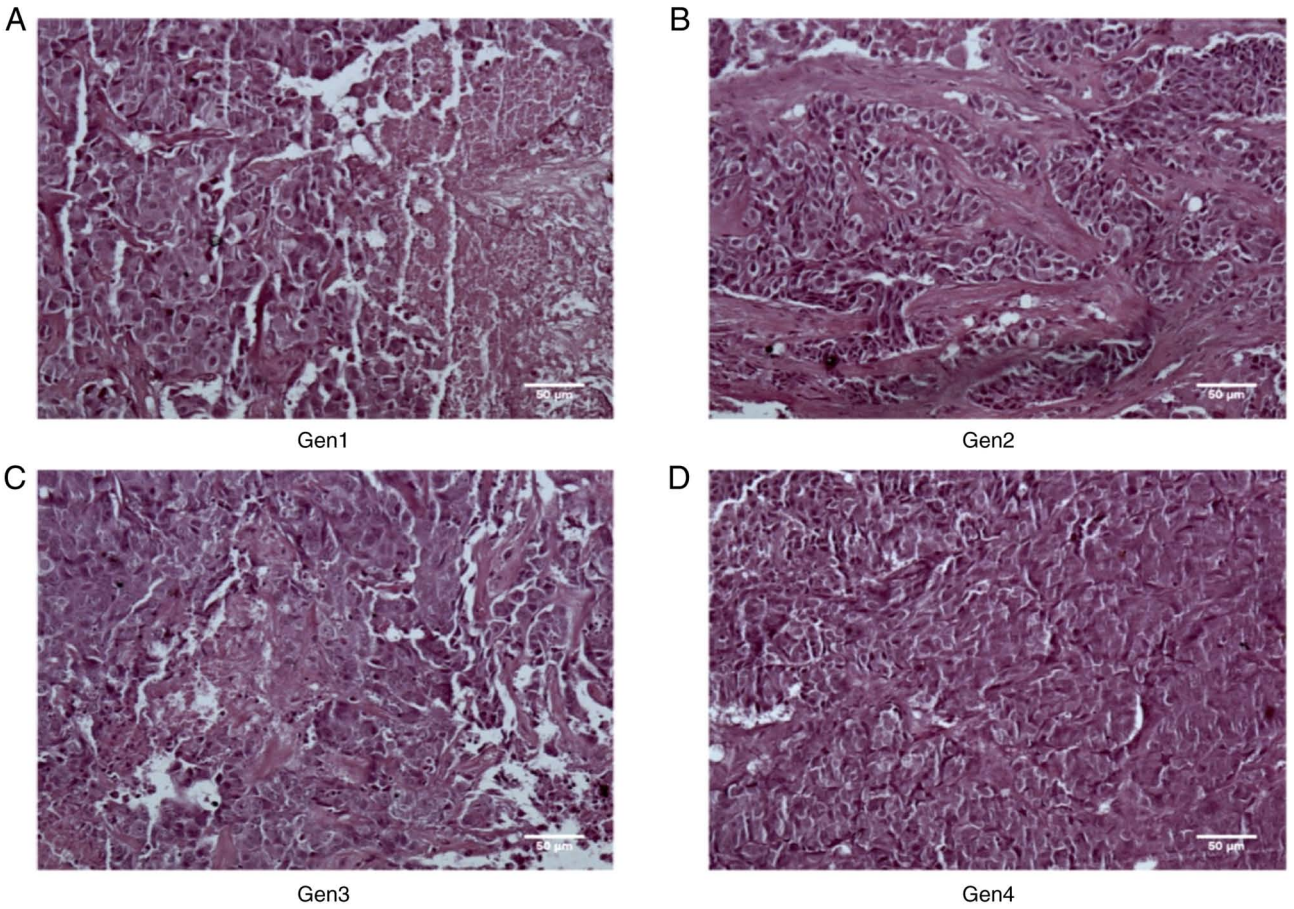

Figure 3. Tumor sections derived from G1 to G4 patient-derived orthotopic xenografts were stained with H&E and images were captured (scale bars, 50 μm). H&E staining of tumor sections indicated similar histological features in (A) generation 1, (B) generation 2, (C) generation 3 and (D) generation 4.

development of specific models for such diseases may allow for personalized treatment and to identify novel molecules that may counter the disease course and improve the outcomes. Hormone receptor-positive, HER2-Neu-negative metastatic breast cancer is one such disease. Currently, there is a lack of preclinical models derived from patients with the aforementioned phenotype.

In the present study, a novel breast PDOX model was successfully developed from a patient with hormone receptor-positive, HER2-Neu-negative metastatic breast cancer who progressed on multiple rounds of therapy. The model was established by directly implanting tumor fragments into the mammary fat pad of an immunocompromised mouse followed by serial passages. An engraftment rate of 20% was noted for generating G1 PDOXs from primary tumors. Previous studies have also reported an engraftment rate of ~20% for the generation of PDXs in NOD/SCID mice (16,17), which may be due to residual activities of natural killer cells in these mice (17,21,22). Different approaches were used to eliminate or suppress these cells and increase the engraftment of the patient's tumors, including the usage of anti-IL2 receptor antibodies or crossbreeding with β-macroglobulin/perforin-deficient mice (17,21). In addition, several studies reported improvements in the engraftment rate by using additional types of immunocompromised mice, such as NOD/Shi-SCID/IL-2Rγ^{null}/NOD

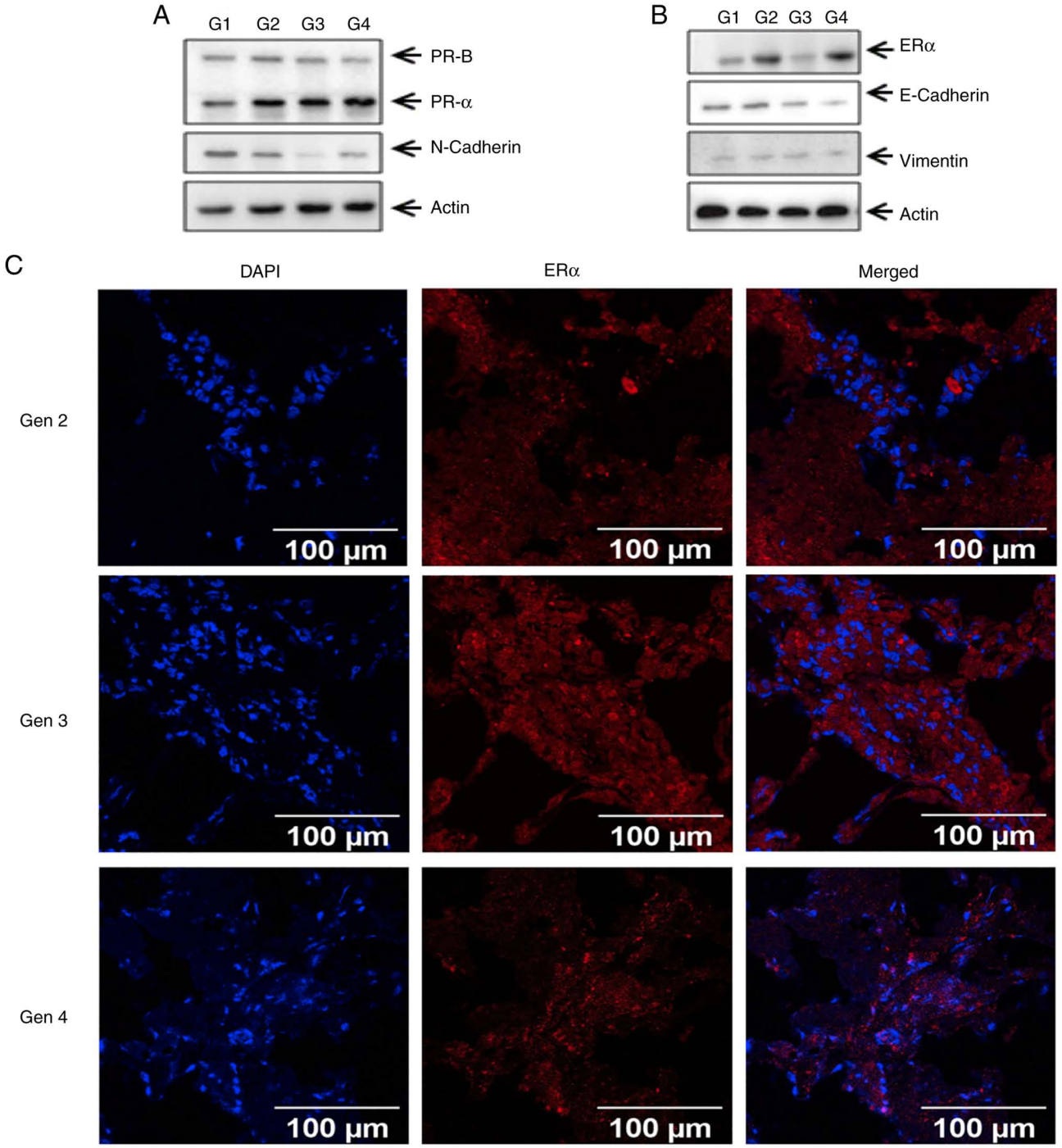

Figure 4. Western blot analysis of breast cancer subtype-specific markers (A) PRa, PRb and epithelial and mesenchymal markers N-cadherin; (B) ERα, E-cadherin and Vimentin in tumor tissues from G1-G4 PDOXs. (C) Immunofluorescence analysis of the level of expression of ERα in PDOXs (G2 to G4) and DAPI for nuclear staining. PDOX, patient-derived orthotopic xenograft; ER, estrogen receptor; PR, progesterone receptor; G/Gen, generation.

SCID gamma (NSG) mouse, SCID/beige and BALB/c background (21,23-25).

Histopathological analysis of the patient's tumors and the PDOXs revealed that these cellular entities have similar morphology and histopathology grade. This is consistent with previous reports that indicate the ability of PDXs to mirror the histopathological features of the patient's tumor (11). However, it was observed that the mitotic activity and necrosis were enhanced following the increased passage number of PDOXs. This is likely due to the patient having received multiple rounds of therapy. Decreased time required for formation of palpable tumors and attaining tumor burden in higher passages of PDOXs explain the increased mitotic activity as noted in these tissues. Chen *et al* (26) reported that the site-specificity of primary tumors is retained in PDOX models. To examine whether the information of the primary tumor is highly preserved in PDOXs, the gene expression profiles of the primary tumor and PDOXs were analyzed using immunofluorescence analysis in primary cultures and western blot analysis of the tumor tissue sections. The data indicated that primary tumors and PDOXs (G1-G4)

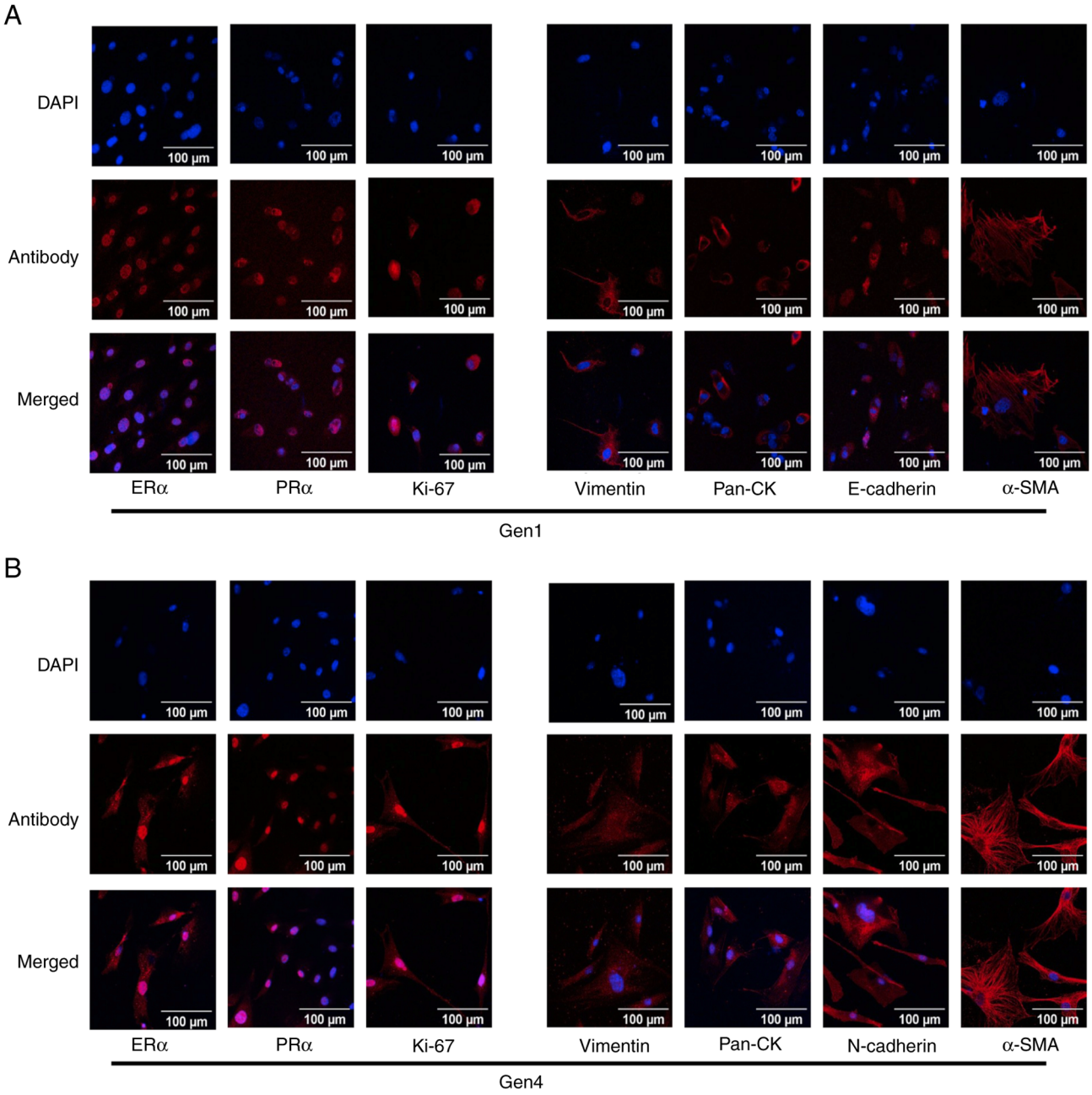

Figure 5. Immunofluorescence analysis of primary culture derived from PDOXs. (A and B) Established primary cultures were seeded on coverslips and fixed. (A) Primary cultures of G1 of PDOXs were stained with breast cancer subtype-specific markers, such as ERα, PRα and Ki-67 and epithelial and mesenchymal markers such as pan-CK, E-cadherin, vimentin and α-SMA. (B) Primary cultures of G4 of PDOXs were stained with breast cancer subtype-specific markers, ERα, PRα and Ki-67 and epithelial and mesenchymal markers such as Pan-CK N-Cadherin, Vimentin and α-SMA. PDOX, patient-derived orthotopic xenograft; SMA, smooth muscle actin; ER, estrogen receptor; PR, progesterone receptor; CK, cytokeratin.

exhibited similar expression profiles of breast-subtype-specific epithelial and mesenchymal markers. This is consistent with the previous reports indicating that PDOXs retain histological and molecular features of the primary tumor. These results suggested that this model is highly reliable for preclinical drug screening and biomarker development. While the present study successfully developed a PDOX model for hormone therapy-resistant breast cancer, the sample number was limited and it was not possible to provide any statistical data for the development of PDOX. Due to the limited sample size of the primary tumor, the present study was confined to histology and IHC and no other comparison studies with PDOX were possible. In the case of the present study, liver metastasis of the primary tumor was confirmed using histology and IHC. However, the lack of metastatic experiments in mice is a limitation of the present study as patient of the present study exhibited liver metastasis. It was also not evaluated whether PDOXs reflect gene expression patterns of primary tumors. Further studies are in progress to determine whether the xenografts recapitulate the mutational burden and gene expression patterns of the primary tumor using next-generation sequencing.

In conclusion, in the present study, a PDOX model for a hormone receptor-positive, HER2/Neu-negative metastatic breast cancer case was successfully developed. The data

indicated that certain histological/molecular features were retained among PDOXs as demonstrated by immunohistochemical, immunofluorescence and western blot analyses. The data also suggest that the developed PDOXs are a reliable pre-clinical model for the further development of novel therapeutics.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

RB, PK, GB and SSM designed and performed most of the experiments. RC, RK, PP and SG devised the patient recruitment strategy, wrote the study protocol and obtained IEC approval. RB, RC, SG and GCK drafted the manuscript. SA, RC, PP, RK, TS, AD and SG acquired clinical samples. SA, RC, PP, RK, TS, AD and SG followed-up patients and acquired the clinical data. RB, PK, GB and SSM developed different generations of PDOX models and established and characterized the primary cultures. TS characterized patient tumor histology and performed IHC on primary patient tumor tissue for ER and PR in the clinical setting. SG and TS analyzed the raw data (H&E staining, ER, PR and HER2 staining) from clinical samples of primary patient tumor at surgical resection and relapse and confirmed the integrity of these data. RB, RC, PP, SG and GCK revised the manuscript draft. GCK conceptualized, designed, supervised the entire work, drafted and corrected the manuscript. AD, SG and GCK checked and confirmed the authenticity of the raw data. All authors have read and approved the manuscript and agreed to be accountable for all aspects of the research. All authors are responsible for the accuracy or integrity of any part of this study.

Ethics approval and consent to participate

The patient was recruited after informed consent under IEC Study 239 (2017) on a protocol approved by Institutional Ethics Committee III of the ACTREC. This study has been registered in the CTRI under the registration no. CTRI/2017/11/010553. All the animal experiments were approved by the Institutional Animal Care and Use Committee of the National Centre for Cell Science (Pune, India; ethics approval no. B-372).

Patient consent for publication

The patient consented to the publication of information about her case presentation/disease course.

Competing interests

The authors declare that they have no competing interests.

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