ANTICANCER RESEARCH *40*: 2509-2514 (2020)
doi:10.21873/anticanres.14221

A Triple-negative Matrix-producing Breast Carcinoma Patient-derived Orthotopic Xenograft (PDOX) Mouse Model Is Sensitive to Bevacizumab and Vinorelbine, Regressed by Eribulin and Resistant to Olaparib

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Abstract. *Background/Aim: Matrix-producing breast carcinoma (MPBC) is a rare and usually aggressive triple-negative breast cancer (TNBC). In this study, we determined drug sensitivity for a triple-negative MPBC, without BRCA mutations, in a patient-derived orthotopic xenograft (PDOX) model. Materials and Methods: The MPBC PDOX model was established in the left 2nd mammary gland of nude mouse by implantation of the patient tumor using surgical orthotopic implantation (SOI). We randomized MPBC PDOX mice into 5 groups (n=5 mice/per treatment group) when the tumor volume reached 80 mm3: G1, control-no treatment; G2, bevacizumab [intra-peritoneal (i.p.), weekly, for 2 weeks]; G3, vinorelbine (i.p., weekly, for 2 weeks); G4, olaparib (oral., daily, for 2 weeks); G5, eribulin [intravenous (i.v.), weekly, for 2 weeks]. The mice in each treatment group were sacrificed on day 15. Tumor volume and body weight were measured once/week. Results: The MPBC PDOX model was resistant to olaparib (p=0.22). The MPBC PDOX model treated with bevacizumab and vinorelbine showed significantly suppressed tumor growth compared to the untreated group (p=0.005 and 0.002, respectively). However, only eribulin regressed the tumor (p=0.0001). Eribulin was more effective than olaparib (p=0.0001), bevacizumab (p=0.0025) and vinorelbine (p=0.0061). Conclusion: Eribulin has clinical potential as treatment for triple-negative MPBC patients that are resistant to a PARP inhibitor such as olaparib.*

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Key Words: PDOX, patient-derived orthotopic xenograft, nude mice, TNBC, triple-negative breast cancer, matrix-producing breast carcinoma, eribulin, olaparib, regression, resistance.

Triple-negative breast cancer (TNBC) lacks expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) protein. Approximately 15 to 20% of all breast cancers are TNBC (1, 2). TNBC is a highly aggressive breast cancer with frequent recurrence and metastasis, and a higher mortality rate compared to other types of breast cancer (3). Furthermore, the

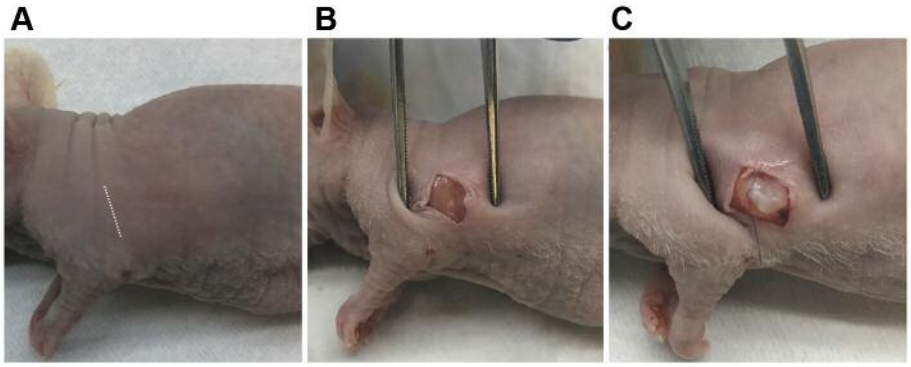

Figure 1. *Surgical orthotopic implantation. (A) 5 mm skin incision was made on the left 2nd mammary gland (white dotted line). (B) The mammary gland was exposed. (C) The mammary gland was incised and a single fragment was implanted at the incised site (10).*

prognosis of the patients with metastatic TNBC (mTNBC) is poor because of the lack of effective targeted therapy (3). Recently, a few therapies have been identified that target a fraction of patients with mTNBC. These include poly ADP ribose polymerase (PARP) inhibitors, olaparib and talazoparib. It has been shown that PARP inhibitors are effective for patients with DNA homologous recombination deficiency (HRD) (specifically in patients with *BRCA1/2* mutations) (4). A recent study showed that PARP inhibitors might also inhibit the growth of cancer cells which do not have DNA HRD (5). Thus, PARP inhibitors are considered first-line for the treatment of TNBC.

Matrix-producing breast carcinoma (MPBC) is a rare, aggressive and specialized subtype of metaplastic breast carcinoma (6). MPBC is mostly a triple-negative and is highly invasive with direct transition to a cartilaginous or osseous matrix with no spindle cells (7, 8). Although effective standardized regimens have been established for TNBC of no special histological type, the efficacy of these treatments, including PARP inhibitors, for minor histological types are unknown because of their rareness (9).

In this study, we compared the efficacy of bevacizumab, vinorelbine and eribulin to olaparib on a patient-derived orthotopic (PDOX) mouse model of triple-negative MPBC.

Materials and Methods

Animal studies. In this study, female athymic *nu/nu* mice (AntiCancer Inc, San Diego, CA, USA), 4-6 weeks old, were used. Animal housing and their diet were based on our previous publications (10). All animals were observed on a daily basis and humanely sacrificed as previously described (10). All animal studies were performed with an AntiCancer Institutional Animal Care and Use Committee (IACUC)-protocol specifically approved for the present study and in accordance with the principles and procedures outlined in the National Institutes of Health (NIH) Guide for the Care and Use of Animals under Assurance Number A3873-1 (10).

Establishment of a triple-negative MPBC PDOX model. A 43-year-old female patient had primary left breast cancer. The patient had a total mastectomy with axillary lymph node dissection at the Kawasaki Medical School Hospital, Japan. The tumor was diagnosed as MPBC without *BRCA* mutations. The results of the immunohistostaining were as follows: ER (–), PgR (–), and HER2 (–). The patient did not receive any neoadjuvant therapy. Written informed consent was obtained from the patient, and the Institutional Ethics Committee of Kawasaki Medical School has approved the PDOX studies. We previously established a PDOX model with the fresh resected tumor specimen which was first implanted subcutaneously in nude mice. The grown subcutaneous tumors were cut into 3 mm^3 fragments for surgical orthotopic implantation (SOI). A 5 mm skin incision was made on the left 2nd mammary gland (Figure 1A). The mammary gland was exposed, and a single fragment was implanted by SOI using 7-0 PDS II (polydioxanone) sutures (Ethicon Inc., Somerville, NJ, USA) (Figure 1B and C). The wound was closed with 5-0 PDS II sutures (Ethicon Inc.) (10, 11).

Treatment protocol for the MPBC PDOX model. The detailed schema of treatments is shown in Figure 2. The MPBC PDOX mice were randomized into five groups (5 mice in each group) when the tumor volume reached 80 mm^3: G1: untreated group; G2: bevacizumab (*i.p.*, 5 mg/kg, weekly 2 weeks); G3: vinorelbine (*i.p.*, 4 mg/kg, weekly, 2 weeks); G4: olaparib (oral., 50 mg/kg, daily, 2 weeks); G5: eribulin (*i.v.*, 1 mg/kg, weekly, 2 weeks). Tumor volume and body weight were measured as previously described (10). All mice were sacrificed on day 15.

Histology. Fresh tumor samples from the mice were fixed, sectioned and stained as described in our previous publication (10). Hematoxylin and eosin (H&E) staining was performed according to the standard protocol. Histological examination was observed with a BHS system microscope (Olympus Corp., Tokyo, Japan) (10).

Statistical analyses. Statistical analyses were conducted with JMP ver. 12.2.0 (SAS Institute, Cary, NC, USA). Comparisons between the 5 groups were determined using one-way ANOVA followed by Tukey *post-hoc* pairwise tests. Bar graphs show the mean, and error bars depict standard error of the mean (SEM). A $p \leq 0.05$ was considered to be statistically significant.

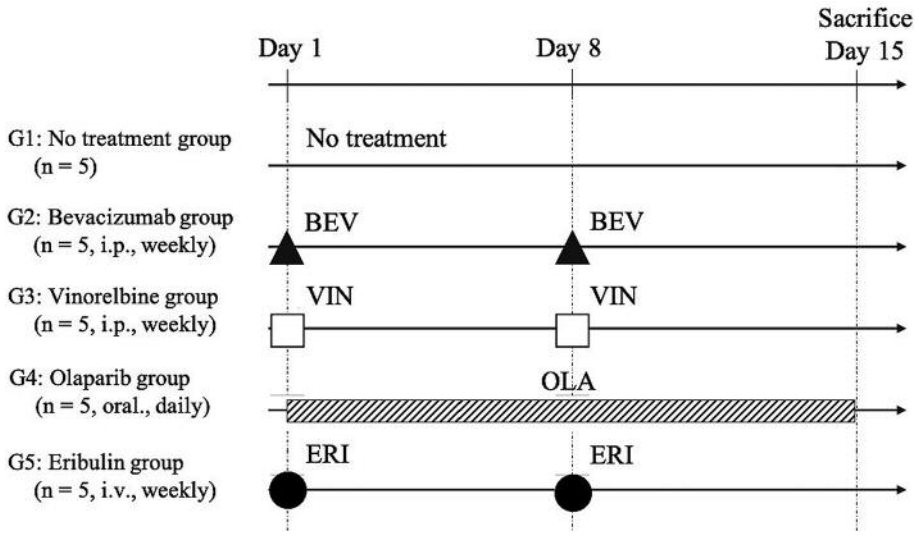

Figure 2. *Schema of treatment protocol.*

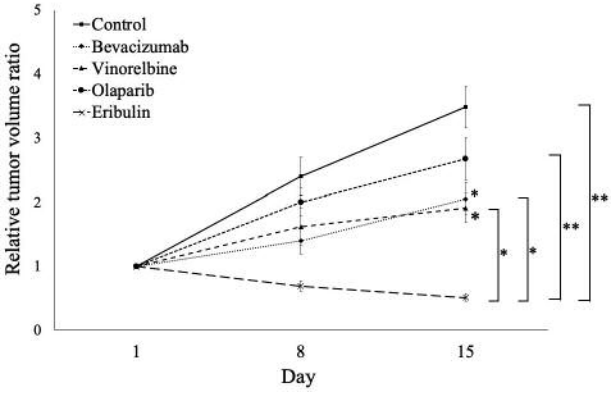

Figure 3. *Quantitative efficacy of chemotherapy on the MPBC PDOX tumor. Line graphs show the relative tumor volume at each time point relative to the start of treatment. Bevacizumab and vinorelbine suppressed tumor growth significantly compared to the control group (p=0.005, p=0.002, respectively). Eribulin regressed tumor growth (p<0.0001) and was more effective compared to other drugs (bevacizumab; p=0.0025, vinorelbine; p=0.0061, olaparib; p=0.0001). *p<0.01. **p<0.001. Error bars: ±SEM.*

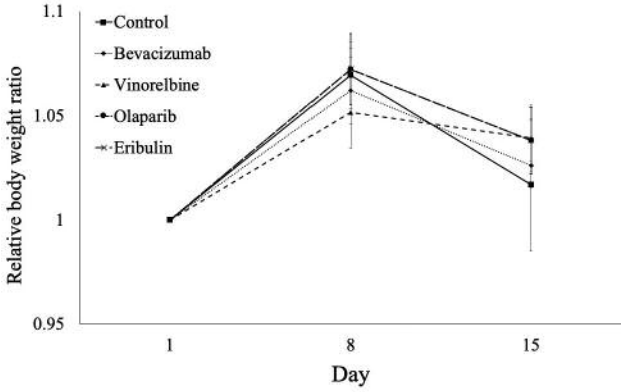

Figure 4. *Relative body weight at each time point relative to the start of treatment. Line graphs illustrate relative body weight in each group on days one, eight and fifteen. Error bars: ±SEM.*

Results

Efficacy of treatments on the MPBC. The efficacy of bevacizumab, vinorelbine, olaparib and eribulin was compared in the MPBC PDOX mouse model. Tumor volume ratios relative to the tumor volume at the start of treatment are shown in Figure 3. The MPBC PDOX model was resistant to olaparib (p=0.22). Bevacizumab and vinorelbine suppressed tumor growth significantly compared to the control group (p=0.005, 0.002, respectively). However, eribulin was the only treatment which regressed the MPBC PDOX tumor (p<0.0001). Eribulin was also significantly more effective compared to olaparib (p=0.0001), bevacizumab (p=0.0025) and vinorelbine (p=0.0061). The final tumor volume ratios were (day 15 *vs*. day 0): the untreated control (G1) (3.49±0.32); bevacizumab-treated (G2) (2.06±0.25); vinorelbine-treated (G3) (1.62±0.23); olaparib-treated (G4) (2.67±0.34); eribulin-treated (G4) (0.51±0.06). These results suggested that eribulin was more efficacious than the other drugs examined in this study and could regress the MPBC PDOX tumor.

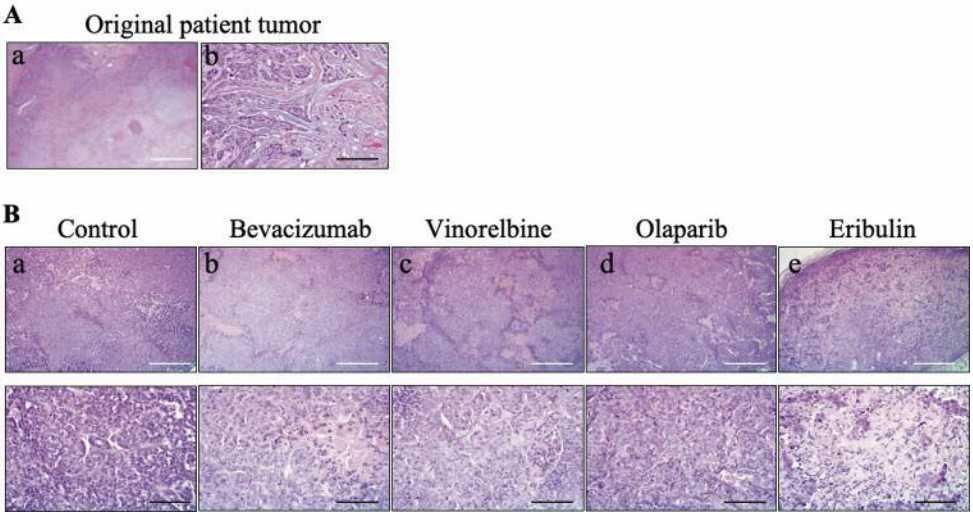

Figure 5. *Tumor histology. (A) Original patient tumor histology. a; low power field (×40), b; high power field (×200). (B) PDOX tumor histology. Hematoxylin and eosin (H&E) staining of the untreated MPBC PDOX tumor (a), MPBC PDOX tumor treated with bevacizumab (b). vinorelbine treated MPBC PDOX tumor (c), olaparib treated MPBC PDOX tumor (d), and eribulin treated MPBC PDOX tumor (e). Upper column: low power field (×40), Lower column: high power field (×200). White scale bar: 500 μm. Black scale bar: 100 μm.*

Body weight. Mouse body weight was measured at pre-treatment, during treatment and post-treatment. We did not find any significant differences in the body weight ratio or body weight loss in any treatment group (Figure 4). These results suggested that the tested doses had no overt side effects. All groups had weight loss after day 8, possibly due to cachexia.

Histology of the MPBC PDOX. Figure 5A shows photomicrographs of H&E-stained sections of the original patient tumor. Figure 5B shows representative photo-micrographs of H&E-stained sections of the PDOX tumor from each group. The control PDOX tumor contained viable highly dense cancer cells. PDOX tumors treated with bevacizumab, vinorelbine or olaparib also contained viable tumor cells, but the cancer-cell densities were lower compared to the untreated control. However, PDOX tumors treated with eribulin had the lowest cancer-cell density, with necrotic areas and degenerative scars in the stroma.

Discussion

This MPBC PDOX was sensitive to eribulin, as well as bevacizumab, vinorelbine, but resistant to olaparib (10, 12, 13). TNBCs are mainly chemotherapy-resistant. Therefore, the prognosis of the patients with mTNBC is poor with a median overall survival (OS) of 13-16 months. A fraction of patients with germline *BRCA1* or *BRCA2* mutation can be treated with PARP inhibitors, such as olaparib and talazoparib (14, 15). In ovarian cancer, PARP inhibitors improved the prognosis of patients without *BRCA* mutations (5).

MPBC is a rare tumor with few reported studies (16, 17). Kusafuka *et al*. have reported the prevalence of MPBCs among all invasive breast cancer cases as only 0.2% (8). MPBC is usually TNBC and has high proliferative activity, indicated by high histological grade, high Ki-67 index, and high levels of p53 expression (6, 7). Shimada *et al*. have reported that the mean Ki-67 index of MPBCs (45%) was higher compared to TNBCs (36%) of no special histological type, suggesting that MPBCs are a biologically aggressive subgroup of TNBC (9).

MPBCs are negative for ER, PR, and HER2, and thus no targeted therapies are currently available, making conventional chemotherapy the backbone of systemic treatment for MPBCs as well as for TNBC of no special histological type. However, because of its rareness, there are only a few studies about treatment for MPBC and the studies have shown that the pathological response of MPBC was poor (9, 17, 18). Therefore, identification of an effective drug is urgently needed for MPBC patients.

We have established PDOX mouse models for all major cancers (11, 19-21), which is more patient-like than subcutaneous patient-derived xenograft (PDX) models (20) and retain the histopathological/molecular characteristics of the original tumor after transplantation in mice (20-22). PDOX models provide a unique opportunity to derive precise and personalized treatment choices for MPBC patients. We developed the first PDOX model of breast cancer in 1993 (11).

The present study shows that the MPBC PDOX was olaparib-resistant even though it is a first-line drug for the disease. In contrast, the MPBC TNBC was sensitive to

bevacizumab and vinorelbine and regressed by eribulin. The MPBC PDOX model should enable precise, individualized, improved therapy for patients with this recalcitrant disease.

Conflicts of Interest

The Authors declare that there are no potential conflicts of interest. AntiCancer, Inc. uses PDOX mouse models for contract research. Y.M., Y.T., T.H., N.S., H.N., S.I, Y.S., H.L., K.M., and RMH are or were unsalaried associates of AntiCancer Inc.

Authors' Contributions

J.Y. and R.M.H designed and performed experiments, analyzed data and wrote the paper; T.M. provided the tumor; N.S., T.H., Y.T., S.I, Y.S., H.L., C.H., K.M., and M.B. gave technical support and conceptual advice. Writing, review, and/or revision of the manuscript: J.Y., R.M.H, I.E., and S.R.S.

Acknowledgements

This paper is dedicated to the memory of A. R. Moossa, M.D., Sun Lee, M.D., Professor Li Jiaxi, and Masaki Kitajima, M.D.

Funding

This work was supported in part by a Yokohama City University research grant "KAMOME Project", which had no role in the design, execution, interpretation, or writing of the study.

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Received March 25, 2020
Revised April 6, 2020
Accepted April 14, 2020