A triple-negative matrix-producing breast carcinoma is arrested by tumor-targeting

***Salmonella typhimurium* A1-R in a PDOX model**

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30

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34 **ABSTRACT**

Background/Aim: Matrix-producing breast carcinoma (MPBC), is a rare, recalcitrant and highly aggressive. The present study aimed to determine the efficacy of tumor-targeting *Salmonella typhimurium* (*S. typhimurium*) A1-R on a triple-negative MPBC in a patient-derived orthotopic xenograft (PDOX) model.

Methods: The PDOX model was established in the left second mammary gland of a nude mouse by surgical orthotopic implantation (SOI) of the patient triple-negative MPBC PDOX models were randomized into two groups: G1, control group (n=6); G2, tumor-targeting *S. typhimurium* A1-R group (n=7, intravenous (i.v.) injection via tail vein, weekly, for two weeks). All mice were sacrificed on day 15. Tumor volume and body weight were measured one time per week.

Results: *S. typhimurium* A1-R arrested tumor growth compared to the control group (P = 0.016).

Conclusion: The results of the present study suggest that *S. typhimurium* A1-R has future clinical potential in triple-negative MPBC patients.

INTRODUCTION

Triple-negative breast cancer (TNBC) is an aggressive and recalcitrant subtype of breast cancer characterized by lack of expression estrogen receptor (ER), progesterone receptor (PgR), and human epidermal growth factor receptor 2 (HER2). TNBC accounts for approximately 15 to 20% of all breast cancer (1, 2). The prognosis of the patients with metastatic TNBC (mTNBC) is particularly poor, due to the lack of effective targeted therapy (3).

Matrix-producing breast carcinoma (MPBC) is a rare and specialized histological type of metaplastic carcinoma (4). MPBC is usually triple-negative breast cancer and defined as an invasive breast carcinoma with a direct transition to a cartilaginous or osseous matrix with no intervening spindle-cell component (5, 6). Effective therapy for triple-negative MPBC has not been established due to its rarity (7).

Our laboratory pioneered the patient-derived orthotopic xenograft (PDOX) model by implanting patient-derived malignant tumor fragments into orthotopic sites in mice 30 years ago by establishing surgical orthotopic implantation (SOI) (8, 9). We have shown that the PDOX model is more patient-like compared to subcutaneous patient-derived xenograft (PDX) models (16). We have previously demonstrated that the PDOX model retains the histopathological/molecular and metastatic characteristics of the original tumor after SOI in

mice (9-11). We previously showed eribulin regressed triple-negative MPBC PDOX mouse models (12, 13).

Salmonella typhimurium (*S. typhimurium*) A1-R is a facultative anaerobe. Green fluorescence protein (GFP)-labeled *S. typhimurium* A1-R (*S. typhimurium* A1-R -GFP), developed by our laboratory, has high tumor-targeting efficacy, due to its leucine–arginine auxotrophy, resulting in broad antitumor efficacy and limited adverse effects (14-16). *S. typhimurium* A1-R-GFP was able to inhibit or eradicate primary and metastatic tumors as monotherapy in nude mouse models of prostate (14, 16), breast (15, 17, 18), lung (19, 20), pancreatic (21-23), ovarian (24, 25), stomach (26), and cervical cancer (27), as well as sarcoma (28-30), melanoma (31), and glioma (32).

The development of new treatment strategies is needed for triple-negative MPBC patients. The present report demonstrates that *S. typhimurium* A1-R-GFP is an effective treatment strategy for triple-negative MPBC in a PDOX mouse model.

MATERIAL AND METHODS

Mouse studies.

In the present study, a thymic *nu/nu* nude female mice (AntiCancer Inc, San Diego, CA), 6-7 weeks old, were used. All mice were kept in a barrier facility on a high efficacy particulate air (HEPA)-filtered rack under standard conditions of 12-h light/dark cycles. Mouse studies were performed with an AntiCancer Institutional Animal Care and Use Committee (IACUC)-protocol specially approved for the present study in accordance with the principles and procedures outlined in the National Institutes of Health Guide for the Care and Use of Animals under Assurance Number A3873-1.

Previous establishment of the triple-negative MPBC PDOX model.

A 43-year-old female patient with primary left breast cancer previously received a total mastectomy with axillary lymph node dissection at Kawasaki Medical School Hospital, Japan. The tumor was diagnosed as matrix-producing carcinoma without *BRCA* mutations. The results of immunohistostaining were as follows: ER (-), PgR (-), HER2 (-). The patient did not receive any neoadjuvant therapy. Informed consent was obtained for the patient's tumor to be established in mouse models for studies, and PDOX studies were approved by the Institutional Ethics Committee of Kawasaki Medical School. A fresh resected tumor specimen was previously implanted subcutaneously in nude mice for

establishment at AntiCancer Japan (33). The established tumors were divided into three mm³ fragments for orthotopic surgical implantation (SOI). A 5 mm skin incision on the left second mammary gland was made under anesthesia. The mammary gland was exposed, and a single fragment was implanted by SOI using 7-0 PDS II (polydioxanone) sutures (Ethicon, Inc., NJ, USA) (12). The wound was closed with 5-0 PDS II sutures (Ethicon, Inc., NJ, USA) (34).

Preparation of S. typhimurium A1-R

GFP-expressing *S. typhimurium* A1-R (AntiCancer Inc.) were grown overnight in LB medium (Fisher Sci., Hanover Park, IL, USA) and then diluted 1:10 in LB medium. Bacteria were harvested in late-log phase, washed with PBS, and then diluted in PBS.

Treatment protocol for the triple-negative MPBC PDOX model.

The detailed experimental schema is shown in Fig. 1. The triple-negative MPBC PDOX models were randomized into two groups when the tumor volume was over 70 mm³: G1: untreated control; G2: *S. typhimurium* A1-R-GFP treatment (i.v., 5 x 10⁷ colony-forming units [CFU] *S. typhimurium* A1-R -GFP in 100 µl phosphate-buffered saline [PBS],

weekly, two weeks). Each group comprised six and seven mice, respectively. Tumor size and body weight were measured once a week. Tumor volume was calculated using the following formula: tumor volume (mm) = length (mm) × width (mm) × width (mm) × 1/2. All mice were sacrificed on day 14.

Fluorescence imaging of the triple-negative MPBC PDOX

Whole-organ images were obtained and the mean fluorescence intensity of organs was determined using the UVP ChemStudio (Analytik Jena, Germany).

Definition of mean fluorescence intensity: Mean raw intensity, minus mean background.

Statistical Analysis.

All statistical analyses were performed with GraphPad Prism 8.4.3 (GraphPad Software, Inc., San Diego, CA). Significant differences for comparisons of two groups were determined using the Student's *t*-test. The experimental data are expressed as the mean ± SD. A probability value of $P \leq 0.05$ was defined as statistically significant.

RESULTS

Selective targeting of the triple-negative MPBC PDOX by S. typhimurium A1-R -GFP

Twenty four hours after infection of triple-negative MPBC PDOX of *S. typhimurium* A1-R -GFP, the bacteria selectively targeted the tumor, as demonstrated by GFP fluorescence, as *S. typhimurium* A1-R -GFP was not detected in the liver or spleen of the triple-negative MPBC PDOX model. (Fig.2).

S. typhimurium A1-R -GFP arrested the triple-negative MPBC PDOX

We tested the efficacy of *S. typhimurium* A1-R on the MPBC PDOX mouse model. *S. typhimurium* A1-R arrested the growth of the triple-negative MPBC (P = 0.016). The final tumor volume ratios were obtained (day 14 vs. day 0): the control group (G1) (2.236 ± 0.556); *S. typhimurium* A1-R -GFP -treated (G2) (1.303 ± 0.533).

No effect of S. typhimurium A1-R -GFP on body weight of the triple-negative MPBC PDOX mouse model

To determine whether the drug treatments had any effect on body weight, we measured the mouse body weight at pre-treatment and post-treatment. The final body weight ratios for (day 14 vs. day 0): for the control group (G1) was 1.031 ± 0.043; and for

the *S. typhimurium* A1-R -GFP -treated (G2) was 0.968 ± 0.093 . There were no significant differences found in the body weight ratio between the two groups on day7, on day14 (P = 0.48, 0.73, respectively) (Fig. 4), which suggests that *S. typhimurium* A1-R -GFP had no obvious side effects.

DISCUSSION

In the present study, *S. typhimurium* A1-R-GFP selectively targeted the triple-negative MPBC PDOX and arrested its growth.

MPBC is a rare tumor with few reported studies. On only a small number of cases (35). Kusafuka et al. reported the frequency of MPBCs among all invasive breast cancer cases as only 0.2% (6). MPBC is usually TNBC and has high proliferative activity, indicated by high histological grade, high Ki-67 index, and high level of p53 expression (4, 5). Shimada et al. reported that the mean Ki-67 index of the MPBCs (45%) was higher than that of TNBCs (36%), suggesting that MPBCs are a biologically aggressive subgroup of TNBC (7).

The triple-negative MPBC is negative for ER, PR, and HER2, the type used in the present study has no approved targeted therapy. Due to its rarely, its rareness, there are only

a few treatment studies for MPBC, they showed poor efficacy (7, 36, 37). Therefore, novel effective therapy is urgently needed for MPBC patients. Our previous studies of triple-negative MPBC PDOX, showed eribulin was effective (12, 13).

Bacteria therapy has gained popularity as a cancer immunotherapy in recent years (38). *Salmonella*, *Clostridium*, and other bacterial genera have been shown to control tumor growth and promote survival in animal models (38). *S. typhimurium* A1-R is an auxotrophic leucine-arginine facultative anaerobic *S. typhimurium* strain which selectively targets and proliferates in tumors of all types due to in part at least its nutritional auxotrophy which appears to be satisfied in the rich nutritional milieu of tumors, which severely restricts its growth in normal tissue (16). In the present study *S. typhimurium* A1-R-GFP selectively localized and proliferated in the triple-negative MPBC tumor. By contrast, *S. typhimurium* A1-R-GFP was undetectable in the liver, spleen. These results suggest the clinical potential of *S. typhimurium* A1-R for triple-negative MPBC.

Conclusion

S. typhimurium A1-R-GFP arrested the growth of a triple-negative MPBC PDOX

model without apparent toxicity. The triple-negative MPBC PDOX model should enable

precise, individualized, improved therapy for patients with this recalcitrant disease.

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204 **References**

- 205 1 Carey LA, Perou CM, Livasy CA, Dressler LG, Cowan D, Conway K, Karaca G,
206 Troester MA, Tse CK, Edmiston S, Deming SL, Geradts J, Cheang MCU, Nielsen
207 TO, Moorman PG, Earp HS and Millikan RC: Race, breast cancer subtypes, and
208 survival in the carolina breast cancer study. *JAMA* 295(21): 2492-2502, 2006.
209 PMID, DOI: 10.1001/jama.295.21.2492
- 210 2 SEER Cancer Statistics Review, 1975–2014. [Internet]. National Cancer Institute. 2017.
- 211 3 Liedtke C, Mazouni C, Hess KR, Andre F, Tordai A, Mejia JA, Symmans WF, Gonzalez-
212 Angulo AM, Hennessy B, Green M, Cristofanilli M, Hortobagyi GN and Puztai L:
213 Response to neoadjuvant therapy and long-term survival in patients with triple-
214 negative breast cancer. *J Clin Oncol* 26(8): 1275-1281, 2008. PMID, DOI:
215 10.1200/jco.2007.14.4147
- 216 4 Wargotz ES and Norris HJ: Metaplastic carcinomas of the breast. I. Matrix-producing
217 carcinoma. *Hum Pathol* 20(7): 628-635, 1989. PMID, DOI: 10.1016/0046-
218 8177(89)90149-4
- 219 5 Gibson GR, Qian D, Ku JK and Lai LL: Metaplastic breast cancer: Clinical features and
220 outcomes. *Am Surg* 71(9): 725-730, 2005. PMID, DOI:

221 6 Kusafuka K, Muramatsu K, Kasami M, Kuriki K, Hirobe K, Hayashi I, Watanabe H,
222 Hiraki Y, Shukunami C, Mochizuki T and Kameya T: Cartilaginous features in
223 matrix-producing carcinoma of the breast: Four cases report with histochemical and
224 immunohistochemical analysis of matrix molecules. *Mod Pathol* 21(10): 1282-
225 1292, 2008. PMID, DOI: 10.1038/modpathol.2008.120

226 7 Shimada K, Ishikawa T, Yamada A, Sugae S, Narui K, Shimizu D, Chishima T and Endo
227 I: Matrix-producing carcinoma as an aggressive triple-negative breast cancer:
228 Clinicopathological features and response to neoadjuvant chemotherapy. *Anticancer*
229 *Res* 39(7): 3863-3869, 2019. PMID, DOI: 10.21873/anticancer.13536

230 8 Fu XY, Besterman JM, Monosov A and Hoffman RM: Models of human metastatic colon
231 cancer in nude mice orthotopically constructed by using histologically intact patient
232 specimens. *Proceedings of the National Academy of Sciences* 88(20): 9345-9349,
233 1991. PMID, DOI: 10.1073/pnas.88.20.9345

234 9 Hoffman RM: Patient-derived orthotopic xenografts: Better mimic of metastasis than
235 subcutaneous xenografts. *Nat Rev Cancer* 15(8): 451-452, 2015. PMID, DOI:
236 10.1038/nrc3972

- 237 10 Furukawa T, Kubota T, Watanabe M, Kitajima M and Hoffman RM: Orthotopic
238 transplantation of histologically intact clinical specimens of stomach cancer to nude
239 mice: Correlation of metastatic sites in mouse and individual patient donors. *Int J*
240 *Cancer* 53(4): 608-612, 1993. PMID, DOI: 10.1002/ijc.2910530414
- 241 11 Hoffman RM: Orthotopic metastatic mouse models for anticancer drug discovery and
242 evaluation: A bridge to the clinic. *Invest New Drugs* 17(4): 343-359, 1999. PMID,
243 DOI: 10.1023/a:1006326203858
- 244 12 Yamamoto J, Murata T, Tashiro Y, Higuchi T, Sugisawa N, Nishino H, Inubushi S, Sun
245 YU, Lim H, Miyake K, Hongo A, Nomura T, Saitoh W, Moriya T, Tanino H,
246 Hozumi C, Bouvet M, Singh SR, Endo I and Hoffman RM: A triple-negative
247 matrix-producing breast carcinoma patient-derived orthotopic xenograft (pdx)
248 mouse model is sensitive to bevacizumab and vinorelbine, regressed by eribulin and
249 resistant to olaparib. *Anticancer Res* 40(5): 2509-2514, 2020. PMID, DOI:
250 10.21873/anticancer.14221
- 251 13 Lim HI, Yamamoto J, Inubushi S, Nishino H, Tashiro Y, Sugisawa N, Han Q, Sun YU,
252 Choi HJ, Nam SJ, Kim MB, Lee JS, Hozumi C, Bouvet M, Singh SR and Hoffman
253 RM: A single low dose of eribulin regressed a highly aggressive triple-negative

254 breast cancer in a patient-derived orthotopic xenograft model. *Anticancer Res* *40*(5):
255 2481-2485, 2020. PMID, DOI: 10.21873/anticancer.14218

256 14 Zhao M, Yang M, Li XM, Jiang P, Baranov E, Li S, Xu M, Penman S and Hoffman
257 RM: Tumor-targeting bacterial therapy with amino acid auxotrophs of gfp-
258 expressing salmonella typhimurium. *Proc Natl Acad Sci U S A* *102*(3): 755-760,
259 2005. PMID: PMC545558, DOI: 10.1073/pnas.0408422102

260 15 Zhao M, Yang M, Ma H, Li X, Tan X, Li S, Yang Z and Hoffman RM: Targeted
261 therapy with a salmonella typhimurium leucine-arginine
262 auxotroph cures orthotopic human breast tumors in nude mice. *Cancer Res* *66*(15):
263 7647, 2006. PMID, DOI: 10.1158/0008-5472.CAN-06-0716

264 16 Zhao M, Geller J, Ma H, Yang M, Penman S and Hoffman RM: Monotherapy with a
265 tumor-targeting mutant of salmonella typhimurium cures
266 orthotopic metastatic mouse models of human prostate cancer. *Proceedings of the*
267 *National Academy of Sciences* *104*(24): 10170, 2007. PMID, DOI:
268 10.1073/pnas.0703867104

269 17 Zhang Y, Tome Y, Suetsugu A, Zhang L, Zhang N, Hoffman RM and Zhao M:
270 Determination of the optimal route of administration of salmonella typhimurium a1-

- 271 r to target breast cancer in nude mice. *Anticancer Res* 32(7): 2501-2508, 2012.
- 272 PMID, DOI:
- 273 18 Zhang Y, Miwa S, Zhang N, Hoffman RM and Zhao M: Tumor-targeting salmonella
- 274 typhimurium a1-r arrests growth of breast-cancer brain metastasis. *Oncotarget* 6(5):
- 275 2615-2622, 2015. PMID: PMC4413605, DOI: 10.18632/oncotarget.2811
- 276 19 Uchugonova A, Zhao M, Zhang Y, Weinigel M, König K and Hoffman RM: Cancer-cell
- 277 killing by engineered salmonella imaged by multiphoton tomography in live mice.
- 278 *Anticancer Res* 32(10): 4331-4337, 2012. PMID, DOI:
- 279 20 Liu F, Zhang L, Hoffman RM and Zhao M: Vessel destruction by tumor-targeting
- 280 salmonella typhimurium a1-r is enhanced by high tumor vascularity. *Cell Cycle*
- 281 9(22): 4518-4524, 2010. PMID: PMC3048048, DOI: 10.4161/cc.9.22.13744
- 282 21 Nagakura C, Hayashi K, Zhao M, Yamauchi K, Yamamoto N, Tsuchiya H, Tomita K,
- 283 Bouvet M and Hoffman RM: Efficacy of a genetically-modified salmonella
- 284 typhimurium in an orthotopic human pancreatic cancer in nude mice. *Anticancer*
- 285 *Res* 29(6): 1873-1878, 2009. PMID, DOI:
- 286 22 Yam C, Zhao M, Hayashi K, Ma H, Kishimoto H, McElroy M, Bouvet M and Hoffman
- 287 RM: Monotherapy with a tumor-targeting mutant of s. Typhimurium inhibits liver

288 metastasis in a mouse model of pancreatic cancer. *J Surg Res* *164*(2): 248-255,
289 2010. PMID: PMC2888721, DOI: 10.1016/j.jss.2009.02.023

290 23 Hiroshima Y, Zhao M, Zhang Y, Maawy A, Hassanein MK, Uehara F, Miwa S, Yano S,
291 Momiyama M, Suetsugu A, Chishima T, Tanaka K, Bouvet M, Endo I and Hoffman
292 RM: Comparison of efficacy of salmonella typhimurium a1-r and chemotherapy on
293 stem-like and non-stem human pancreatic cancer cells. *Cell Cycle* *12*(17): 2774-
294 2780, 2013. PMID: PMC3899191, DOI: 10.4161/cc.25872

295 24 Matsumoto Y, Miwa S, Zhang Y, Hiroshima Y, Yano S, Uehara F, Yamamoto M,
296 Toneri M, Bouvet M, Matsubara H, Hoffman RM and Zhao M: Efficacy of tumor-
297 targeting salmonella typhimurium a1-r on nude mouse models of metastatic and
298 disseminated human ovarian cancer. *J Cell Biochem* *115*(11): 1996-2003, 2014.
299 PMID, DOI: 10.1002/jcb.24871

300 25 Matsumoto Y, Miwa S, Zhang Y, Zhao M, Yano S, Uehara F, Yamamoto M, Hiroshima
301 Y, Toneri M, Bouvet M, Matsubara H, Tsuchiya H and Hoffman RM:
302 Intraperitoneal administration of tumor-targeting salmonella typhimurium a1-r
303 inhibits disseminated human ovarian cancer and extends survival in nude mice.

304 Oncotarget *6(13)*: 11369-11377, 2015. PMID: PMC4484462, DOI:
305 10.18632/oncotarget.3607

306 26 Yano S, Zhang Y, Zhao M, Hiroshima Y, Miwa S, Uehara F, Kishimoto H, Tazawa H,
307 Bouvet M, Fujiwara T and Hoffman RM: Tumor-targeting salmonella typhimurium
308 a1-r decoys quiescent cancer cells to cycle as visualized by fucci imaging and
309 become sensitive to chemotherapy. *Cell Cycle* *13(24)*: 3958-3963, 2014. PMID:
310 PMC4615054, DOI: 10.4161/15384101.2014.964115

311 27 Hiroshima Y, Zhang Y, Zhao M, Zhang N, Murakami T, Maawy A, Mii S, Uehara F,
312 Yamamoto M, Miwa S, Yano S, Momiyama M, Mori R, Matsuyama R, Chishima
313 T, Tanaka K, Ichikawa Y, Bouvet M, Endo I and Hoffman RM: Tumor-targeting
314 salmonella typhimurium a1-r in combination with trastuzumab eradicates her-2-
315 positive cervical cancer cells in patient-derived mouse models. *PLoS One* *10(6)*:
316 e0120358, 2015. PMID: PMC4457918, DOI: 10.1371/journal.pone.0120358

317 28 Murakami T, DeLong J, Eilber FC, Zhao M, Zhang Y, Zhang N, Singh A, Russell T,
318 Deng S, Reynoso J, Quan C, Hiroshima Y, Matsuyama R, Chishima T, Tanaka K,
319 Bouvet M, Chawla S, Endo I and Hoffman RM: Tumor-targeting salmonella
320 typhimurium a1-r in combination with doxorubicin eradicate soft tissue sarcoma in

321 a patient-derived orthotopic xenograft (pdx) model. *Oncotarget* 7(11): 12783-
322 12790, 2016. PMID: PMC4914321, DOI: 10.18632/oncotarget.7226

323 29 Hiroshima Y, Zhao M, Zhang Y, Zhang N, Maawy A, Murakami T, Mii S, Uehara F,
324 Yamamoto M, Miwa S, Yano S, Momiyama M, Mori R, Matsuyama R, Chishima
325 T, Tanaka K, Ichikawa Y, Bouvet M, Endo I and Hoffman RM: Tumor-targeting
326 salmonella typhimurium a1-r arrests a chemo-resistant patient soft-tissue sarcoma in
327 nude mice. *PLoS One* 10(8): e0134324, 2015. PMID: PMC4523197, DOI:
328 10.1371/journal.pone.0134324

329 30 Kiyuna T, Murakami T, Tome Y, Kawaguchi K, Igarashi K, Zhang Y, Zhao M, Li Y,
330 Bouvet M, Kanaya F, Singh A, Dry S, Eilber FC and Hoffman RM: High efficacy
331 of tumor-targeting salmonella typhimurium a1-r on a doxorubicin- and dactolisib-
332 resistant follicular dendritic-cell sarcoma in a patient-derived orthotopic xenograft
333 pdx nude mouse model. *Oncotarget* 7(22): 33046-33054, 2016. PMID:
334 PMC5078074, DOI: 10.18632/oncotarget.8848

335 31 Yamamoto M, Zhao M, Hiroshima Y, Zhang Y, Shurell E, Eilber FC, Bouvet M, Noda
336 M and Hoffman RM: Efficacy of tumor-targeting salmonella a1-r on a melanoma

337 patient-derived orthotopic xenograft (pdx) nude-mouse model. PLoS One 11(8):
338 e0160882, 2016. PMID: PMC4976963, DOI: 10.1371/journal.pone.0160882

339 32 Momiyama M, Zhao M, Kimura H, Tran B, Chishima T, Bouvet M, Endo I and
340 Hoffman RM: Inhibition and eradication of human glioma with tumor-targeting
341 salmonella typhimurium in an orthotopic nude-mouse model. Cell Cycle 11(3): 628-
342 632, 2012. PMID: PMC3315098, DOI: 10.4161/cc.11.3.19116

343 33 Murata T, Hozumi C, Hiroshima Y, Shimoya K, Hongo A, Inubushi S, Tanino H and
344 Hoffman RM: Co-implantation of tumor and extensive surrounding tissue improved
345 the establishment rate of surgical specimens of human-patient cancer in nude mice:
346 Toward the goal of universal individualized cancer therapy. In Vivo 34(6): 3241-
347 3245, 2020. PMID, DOI: 10.21873/invivo.12160

348 34 Fu X, Le P and Hoffman RM: A metastatic orthotopic-transplant nude-mouse model of
349 human patient breast cancer. Anticancer Res 13(4): 901-904, 1993. PMID, DOI:

350 35 Ayar S, Dyess DL and Carter E: Matrix-producing carcinoma: A rare variant of
351 metaplastic breast carcinoma with heterologous elements. Breast J 16(4): 420-423,
352 2010. PMID, DOI: 10.1111/j.1524-4741.2010.00925.x

353 36 Al Sayed AD, El Weshi AN, Tulbah AM, Rahal MM and Ezzat AA: Metaplastic
354 carcinoma of the breast clinical presentation, treatment results and prognostic
355 factors. *Acta Oncol* 45(2): 188-195, 2006. PMID, DOI:
356 10.1080/02841860500513235

357 37 Hennessy BT, Giordano S, Broglio K, Duan Z, Trent J, Buchholz TA, Babiera G,
358 Hortobagyi GN and Valero V: Biphasic metaplastic sarcomatoid carcinoma of the
359 breast. *Ann Oncol* 17(4): 605-613, 2006. PMID, DOI: 10.1093/annonc/mdl006

360 38 Forbes NS, Coffin RS, Deng L, Evgin L, Fiering S, Giacalone M, Gravekamp C, Gulley
361 JL, Gunn H, Hoffman RM, Kaur B, Liu K, Lyerly HK, Marciscano AE, Moradian
362 E, Ruppel S, Saltzman DA, Tattersall PJ, Thorne S, Vile RG, Zhang HH, Zhou S
363 and McFadden G: White paper on microbial anti-cancer therapy and prevention.
364 *Journal for ImmunoTherapy of Cancer* 6(1): 78, 2018. PMID, DOI:
365 10.1186/s40425-018-0381-3

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370 Figure Legends

371 Figure 1

372 Experimental schema. *S. typhimurium* A1-R-GFP was injected into the tail vein of a MPBC
373 TNBC PDOX nude mouse (5×10^7 colony-forming units (cfu) per 100 μ l of PBS). SOI:
374 orthotopic surgical implantation. Please see Materials and Methods for details.

375

376 Figure 2

377 Tumor targeting by *S. typhimurium* A1-R-GFP

378 Selective tumor targeting of *S. typhimurium* A1-R-GFP to the triple-negative MPBC

379 PDOX. Imaging was performed with the UVP ChemStudio (Analytik Jena, Germany).

380 Please see Materials and Methods for details.

381

382 Figure 3

383 Efficacy of *S. typhimurium* A1-R-GFP on the triple-negative MPBC PDOX nude mice.

384 Line graphs show the tumor volume ratio compare to day 0 from initiation of therapy.

385 Tumors were measured at the indicated time points after the initiation of treatment. Control

group n=6 vs. *S. typhimurium* A1-R-GFP treated group n=7. Please see materials and

methods for detail. $P = 0.016$. Error bars represent the mean $\pm$ SD.

Figure 4

Effect of *S. typhimurium* A1-R-GFP on mouse body weight ratio in each group.

Line graphs show the body weight ratio of mice. No significant body-weight differences

were observed between the groups at the each point. Control group n=6 vs., *S. typhimurium*

A1-R-GFP treated group n=7, on day7, on day14, $P = 0.48, 0.73$, respectively.

Figure 1

Figure 2

Figure 3

Figure 4

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