Salmonella typhimurium A1-R Exquisitely Targets and Arrests a Matrix-producing Triple-negative Breast Carcinoma in a PDOX Model

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Abstract. Background/Aim: Triple-negative matrix-producing breast carcinoma (MPBC) is rare, recalcitrant, and highly aggressive. The present study aimed to determine the efficacy of tumor-targeting leucine-arginine auxotroph Salmonella typhimurium (S. typhimurium) A1-R on a triple-negative MPBC in a patient-derived orthotopic xenograft (PDOX) model. Materials and Methods: The PDOX MPBC model was established in the left second mammary gland of nude mice by surgical orthotopic implantation (SOI). PDOX models were randomized into two groups when the tumor volume reached over 70 mm3: a control group (n=6); and a tumor-targeting S. typhimurium A1-R group (n=7), [intravenous (i.v.) injection of S. typhimurium A1-R via the tail vein, weekly, for two weeks]. All mice were sacrificed on day 14. Tumor volume and body weight were measured once per week. Results: S. typhimurium A1-R exquisitely targeted and arrested the growth of the MPBC PDOX compared to the control group (p=0.017). Conclusion: S. typhimurium A1-R has future clinical potential for triple-negative MPBC patients.

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**Results**

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

**Efficacy of S. typhimurium A1-R-GFP on the triple-negative MPBC PDOX.** *S. typhimurium* A1-R arrested the growth of the triple-negative MPBC (*p* = 0.017). The final tumor volume was obtained on treatment-day 14. Average tumor volume of the control group was 205 ± 70 mm³ and in the *S. typhimurium* A1-R-GFP group 112 ± 37 mm³ (Figure 3).

**Discussion**

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

Kusafuka et al. reported the frequency of MPBCs is only 0.2% of all breast cancers (2). MPBC is usually triple-negative breast cancer (TNBC) and has a high proliferative activity, indicated by high histological grade, high Ki-67 index, and high level of p53 expression (1, 29). Shimada et al. reported the mean Ki-67 index of non-TNBC MPBCs (45%) was higher than that of TNBCs (36%) (3).

Due to its rarity, there are only a few clinical trials for MPBC and they showed poor efficacy (3, 30, 31). Therefore, novel effective therapy is urgently needed for MPBC patients. Our previous studies of triple-negative MPBC and common triple-negative breast cancer (TNBC) PDOX mouse models showed eribulin was effective (8, 9).

Bacterial therapy has gained popularity as a cancer immunotherapy in recent years (32). *Salmonella, Clostridium,* and other bacterial genera have been shown to control tumor growth and promote survival in animal models (32). *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, at least in part, its nutritional needs, which appear to be satisfied in...
Figure 2. Tumor targeting by *S. typhimurium* A1-R-GFP. Selective tumor targeting of *S. typhimurium* A1-R-GFP to the triple-negative matrix-producing breast carcinoma (MPBC) PDOX. Imaging was performed with the UVP ChemStudio (Analytik, Jena, Germany). Representative images are shown.

Figure 3. Efficacy of *S. typhimurium* A1-R-GFP on the triple-negative matrix-producing breast carcinoma (MPBC) PDOX nude mice. 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). Line graphs show the tumor volume at the indicated time points. Error bars represent the mean±SD.

Figure 4. Effect of *S. typhimurium* A1-R-GFP on mouse body weight in each group. Bar graphs show the bodyweight of mice at the indicated time points. No significant body weight differences were observed between the groups at each point. Control group (n=6) vs. *S. typhimurium* A1-R-GFP-treated group (n=7), on day 0, on day 14, p=0.53, 0.73, respectively.
the rich nutritional milieu of tumors, but it is severely restricted in normal tissue (12). Thus *S. typhimurium* A1-R can directly target tumors as well as serve as an anti-tumor immuno-stimulator. In a previous study, we showed that *S. typhimurium* A1-R enabled CD-8 T-cells to penetrate tumors (33). In the present study, *S. typhimurium* A1-R-GFP primarily localized and proliferated in the triple-negative MPBC tumor and *S. typhimurium* A1-R-GFP was undetectable in the liver and spleen. These results suggest the clinical potential of *S. typhimurium* A1-R for triple-negative MPBC.

**Conclusion**

*S. typhimurium* A1-R-GFP exquisitely targeted and arrested the growth of a triple-negative MPBC in a PDOX mouse model without apparent toxicity. The triple-negative MPBC PDOX model allows the development of precise, individualized, improved therapy for patients with this recalcitrant disease.

**Conflicts of Interest**

AntiCancer Inc. uses PDOX models for contract research. K.H., Y.A., M.Z., N.S., and R.M.H. are or were unsalaried associates of AntiCancer Inc. C.H. is an unsalaried associate of AntiCancer Japan. The Authors declare no competing financial interests.

**Authors’ Contributions**

K.H. and R.M.H designed and performed experiments, analyzed data, and wrote the article; T.M. provided the tumor specimen; C.H. established the patient tumor specimens in nude mice; J.Y., Z.M., N.S., Y.A., and M.B. provided technical support and conceptual advice; K.H., TT, and R.M.H. wrote, reviewed, and/or revised the manuscript.

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**References**


