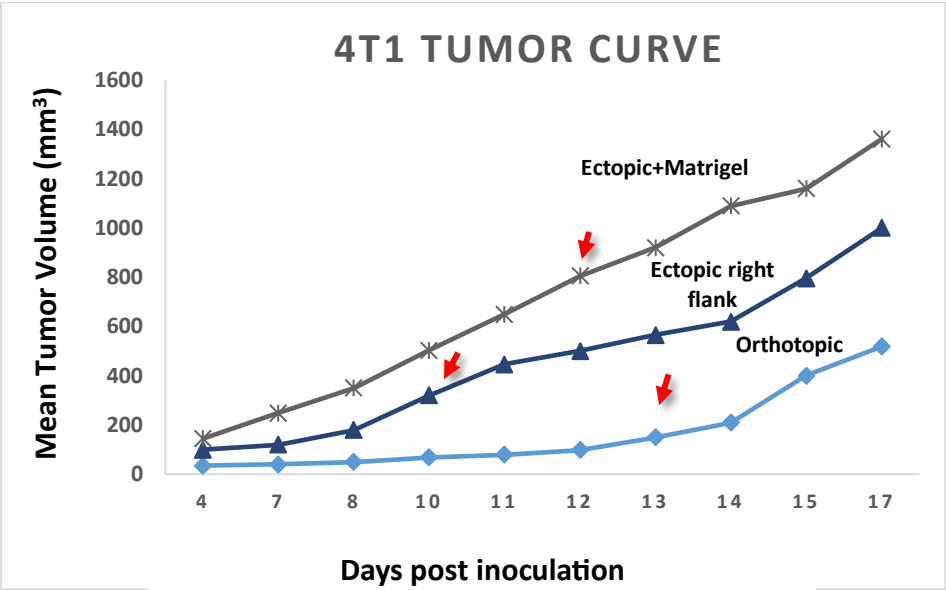
Breast cancer: 4T1 Syngeneic Mouse Model

Introduction Breast cancer is one of the leading causes of death in women across the globe. Although significant advances in research, diagnosis and treatment of the disease have been made in the last decades, it still remains a major health concern. In 2022 approximately 2.3 million women diagnosed with breast cancer globally and 670,000 of them died[1]. Although age is a predisposing factor, young women are not "immune" to the disease. Women under the age of 40 account for the 5-7% of all breast cancers making it the highest diagnosed cancer for this age group[2]. The modest improvements in treating the disease by established therapeutic methods have led both the scientific community and pharmaceutical industry in a quest of new approaches. Animal models are an invaluable tool for testing the efficacy and safety of these novel approaches.

Mice are commonly used as induced models, in which an allograft or xenograft is transplanted either ectopically or orthotopically. In breast cancer research an advantageous model is the transplantation of 4T1 cells in BALB/c mice. The 4T1 cell line is one of the four sublines derived from a spontaneous mammary tumor observed in a BALB/cfC3H mouse and is highly tumorigenic in normal, syngeneic hosts, yielding tumors of generally similar histology, although distinct from the original neoplasm[3]. Furthermore, it is invasive and, unlike most tumor models, can spontaneously metastasize from the primary tumor in the mammary gland to multiple distant sites including lymph nodes, blood, liver, lung, brain, and bone and is 6-thioguanine resistant[4]. Significant advantages of the 4T1 cell line are: they are relatively easily manipulated *in vitro* and *in vivo*, they can be transplanted orthotopically (more closely mimicking the human condition), they are able to invade and metastasise (allowing for modeling a comparable condition to humans after surgical excision of the primary tumor), and they are resistant to 6-thioguanine which permits a more precise quantitation of metastatic cells, even at sub-microscopic levels even at distant organs).

In Bioemtech's facility, experience has been acquired on three models of 4T1 cell line syngeneic mouse model, through ectopic and orthotopic transplantation. After induction and maintenance of anaesthesia with isoflurane, BALB/c female mice (7 to 12 weeks old) are inoculated with 4T1 cells, either in the right flank (1 X 10^6) or on the region of the right acromion (with 1 X 10^5 mixed with Corning® Matrigel® Basement Membrane Matrix cells in the subcutaneous tissue) ectopically or into the 4^{th} right mammary gland (1 X 10^4 cells) orthotopically. The size of the tumor is evaluated with an electronic caliper. The curve of the tumor's volume is represented in the figure. For the orthotopic inoculation ulceration was observed on the 13^{th} day, while for the ectopic and ectopic plus Matrigel on the 10^{th} and 12^{th} day respectively (as indicated by the red arrows).



Studying cancer development and metastasis was commonly performed on immunosuppressed animals using xenografts. This approach is no longer preferable due to advancements in immunotherapies and the need to understand the more complex mechanisms impacting their efficacies. A more advisable model for the appropriate approximation would be the inoculation of tumor cells into a syngeneic immunocompetent animal such as the 4T1 mouse model for breast cancer studies[5].

Luciferase expression through transduction with lentiviral vector of 4T1 cells, encoding firefly luciferase gene has been used as a means for better *in vivo* bioluminescence imaging of mouse model of breast cancer, but at the same time elicits immune responses from the host animals revealing a mechanism of resistance which may be of interest.

Similarly, although 4T1 mouse model is not appropriate for the study of hormone positive, or Human Epidermal growth factor Receptor (HER) 2 positive breast cancers, constructs may be transfected to 4T1 cells in order to express HER2 and evaluate therapies[6, 7].

Furthermore, monoclonal antibodies, or nano-delivered drugs targeting specific features of the pathophysiology of the tumor, can be investigated on the 4T1 mouse model and provide the necessary clues for different pharmacological pathways which may deliver more efficient therapies and therapeutic approaches against breast cancer[8, 9].

1. www.bcrf.org/breast-cancer-statistics-and-resources/
2. Reyna C, Lee MC. Breast cancer in young women: special considerations in multidisciplinary care. J Multidiscip Healthc. 2014 Sep 29;7:419-29. doi: 10.2147/JMDH.S49994. PMID: 25300196; PMCID: PMC4189712.
3. (Dexter DL, Kowalski HM, Blazar BA, Fligiel Z, Vogel R, Heppner GH. Heterogeneity of tumor cells from a single mouse mammary tumor. Cancer Res. 1978 Oct;38(10):3174-81. PMID: 210930.

4. Lelekakis, M., Moseley, J., Martin, T., Hards, D., Williams, E., Ho, P., Lowen, D., Javni, J., Miller, F., Slavin, J., and Anderson, R. 1999. A novel orthotopic model of breast cancer metastasis to bone. Clin. Exp. Metastasis 17:163-170.
5. Grzelak CA, Ghajar CM. Elimination of 4T1 Mammary Tumor Cells by BALB/cBy UBC-GFP Transgenics following Stable Inheritance of the H-2b MHC Allele. Immunohorizons. 2023 Jan 1;7(1):64-70. doi: 10.4049/immunohorizons.2200101. PMID: 36637515; PMCID: PMC10563439.
6. Bakhtiarvand, Vahid Khaki; Akbari, Khadijeh Ramezani-Ali; Sadri, Fatemeh1; Haghighat, Farzaneh Notash; Hojjat-Farsangi, Mohammad; Shokri, Fazel; Jeddi-Tehrani, Mahmood; Shabani, Mahdi. Establishment of a murine model of breast cancer expressing human epidermal growth factor receptor 2
7. Li P, Yang L, Li T, Bin S, Sun B, Huang Y, Yang K, Shan D, Gu H, Li H. The Third Generation Anti-HER2 Chimeric Antigen Receptor Mouse T Cells Alone or Together With Anti-PD1 Antibody Inhibits the Growth of Mouse Breast Tumor Cells Expressing HER2 *in vitro* and in Immune Competent Mice. Front Oncol. 2020 Jul 14;10:1143. doi: 10.3389/fonc.2020.01143. PMID: 32766150; PMCID: PMC7381237.
8. Stagg J, Divisekera U, McLaughlin N, Sharkey J, Pommey S, Denoyer D, Dwyer KM, Smyth MJ. Anti-CD73 antibody therapy inhibits breast tumor growth and metastasis. Proc Natl Acad Sci U S A. 2010 Jan 26;107(4):1547-52. doi: 10.1073/pnas.0908801107. Epub 2010 Jan 4. PMID: 20080644; PMCID: PMC2824381.
9. Zhao Y, Tan Y, Meng T, Liu X, Zhu Y, Hong Y, Yang X, Yuan H, Huang X, Hu F. Simultaneous targeting therapy for lung metastasis and breast tumor by blocking the NF-κB signaling pathway using Celastrol-loaded micelles. Drug Deliv. 2018 Nov;25(1):341-352. doi: 10.1080/10717544.2018.1425778. PMID: 29355035; PMCID: PMC6058533.