



5/24/2019

Dear JoVE Editor:

We are submitting our manuscript, entitled **"A Practical Guide to Study Triple Negative Breast Cancer Using Orthotopic Breast Cancer Model"** to be considered for publication in JoVE. The manuscript is an original work, has not been previously published, and is not under consideration for publication elsewhere.

We previously utilized the orthotopic breast cancer model for our breast cancer metastasis studies (Basudhar *et al* J Med Chem 2013; Heinecke *et al* PNAS 2014, Cheng *et al* Nitric Oxide 2014; Basudhar *et al* *FRBM* 2015). In this manuscript, we provide detailed steps of establishing an orthotopic breast cancer model. We demonstrate the practical guidance for cell line preparation, tumor burden measurement, in vivo imaging, ex vivo imaging, metastatic lung lesion assessment, and molecular detection/validation. In addition, we provide suggestions on validation of imaging data, data analysis as well as data interpretation. This manuscript is able to guide the researchers who are interested in setting up an orthotopic breast cancer model for the assessment of therapeutic efficacy. Overall, it helps in developing therapeutic agents against breast cancer especially metastasis.

All authors have read the manuscript, agreed that the work is ready for submission to JoVE, and accepted the responsibility for the manuscript's contents. We declare that we have no competing financial interests.

Thank you for considering our manuscript for publication in the JoVE.

Yours sincerely,

A handwritten signature in black ink, appearing to read "Robert Cheng".

Dr. Y.S. Robert Cheng, D.V.M., Ph.D.
Molecular Mechanisms Section,
Cancer and Inflammation Program,
Center for Cancer Research,
National Cancer Institute,
Office: Building 576 Room 109
Lab: Building 567 Room 275
1050 Boyles Street,
Frederick, MD 21702-1201
Phone: (301) 228-4386