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### ▶ To cite this version:

Chloé Jean, Lucille Cartier, Christian Garbar, Clémence Fleury, Stéphane Potteaux, et al.. Development of cellular model to explore the 18FES binding in breast cancers with ER mutations by  $\mu$ PET imaging. OncoTrans 2022 & 4th International Liquid Biopsy Symposium, 2022, Nancy, France. hal-04755205

## HAL Id: hal-04755205 https://hal.science/hal-04755205v1

Submitted on 27 Oct 2024

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follow-up











# Development of cellular model to explore the 18FES binding in breast cancers with ER mutations by µPET imaging.

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# Cancer cell cell accorded acco

Breast cancer is the most common cancer in the world and the most deadly in women in France. The ER+ (Estrogen Receptor +) subtype accounts for 70% of breast cancers and is associated with the best overall survival at 5 years. However, in case of recurrence, this duration falls (9 months to 3 years), and tumor cells develop mutations that lead to constitutive activation of the alpha receptor to estrogen in 10 to 50% of cases.

Positron emission tomography (PET) is widely used for diagnosis and for assessing response to therapeutic treatment. The 18Fluorodeoxyglucose (18FDG) radioisotope is a glucose-like tracer commonly used for tumor detection, but unable to provide information on the ER status. The use of 18FES, a compound akin to estradiol should solve this problem and aim to develop personalized therapeutic treatment.

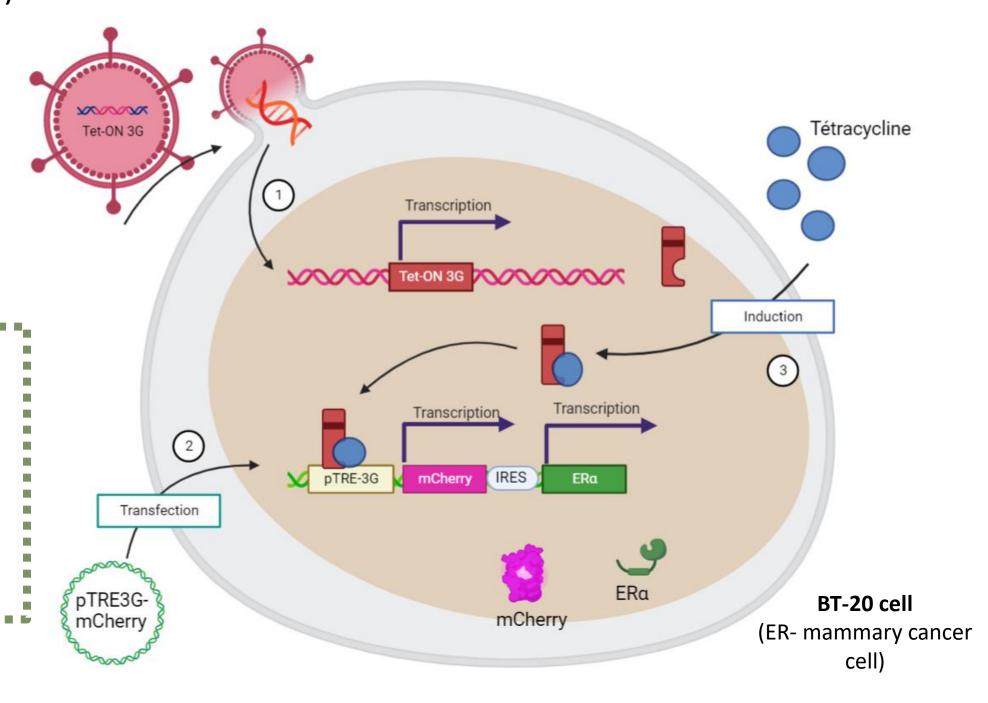
However, its ability to bind on ER mutants is not yet elucidated.

## GOAL

Studying the ability of 18FES to bind on the different ER mutants (E380Q, L536H, Y537C/N/S and D538G) aiming to propose it use for the diagnosis and the monitoring of ER breast cancers.

## FROM CREATING A MODEL ...

- **1. Transduction** of a vector (Tet-ON 3G) allowing stable expression of the transactivator protein Tet-ON 3G.
- **2. Stable transfection** of a vector (pTRE3G-mCherry) allowing the expression of the WT or mutated  $ER\alpha$  and a reporter gene encoding a fluorescent protein (mCherry).
- **3. Induction** of the system by addition of tetracycline (or doxycycline), which by binding to the Tet-on 3G protein, will activate the transcription of genes encoding proteins of interest (ER $\alpha$  and mCherry).



## ..... TO THE IN VITRO...

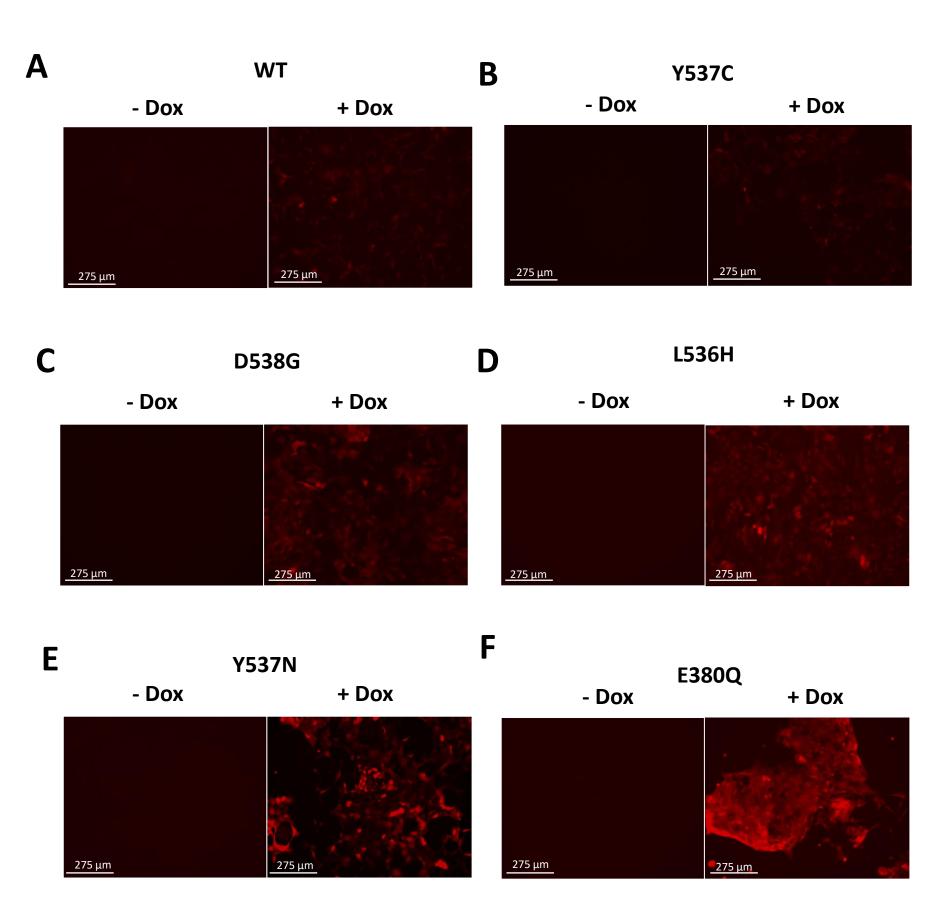


Figure 1: Study of the inducible character of the expression of the mCherry reporter gene expression by fluorescence microscopy. BT-20 cells expressing the transactivator and pTRE3G-mCherry vector containing ERα WT or its mutated variants were stimulated or not with 1000 ng/ml doxycycline (Dox) for 24 hours. Cells expressing ERα WT (A), Y537C (B), D538G (C), L536H (D), Y537N (E) and E380Q (F) were observed and photographed under a fluorescence microscope (Evos Thermofisher Fl Auto 2).

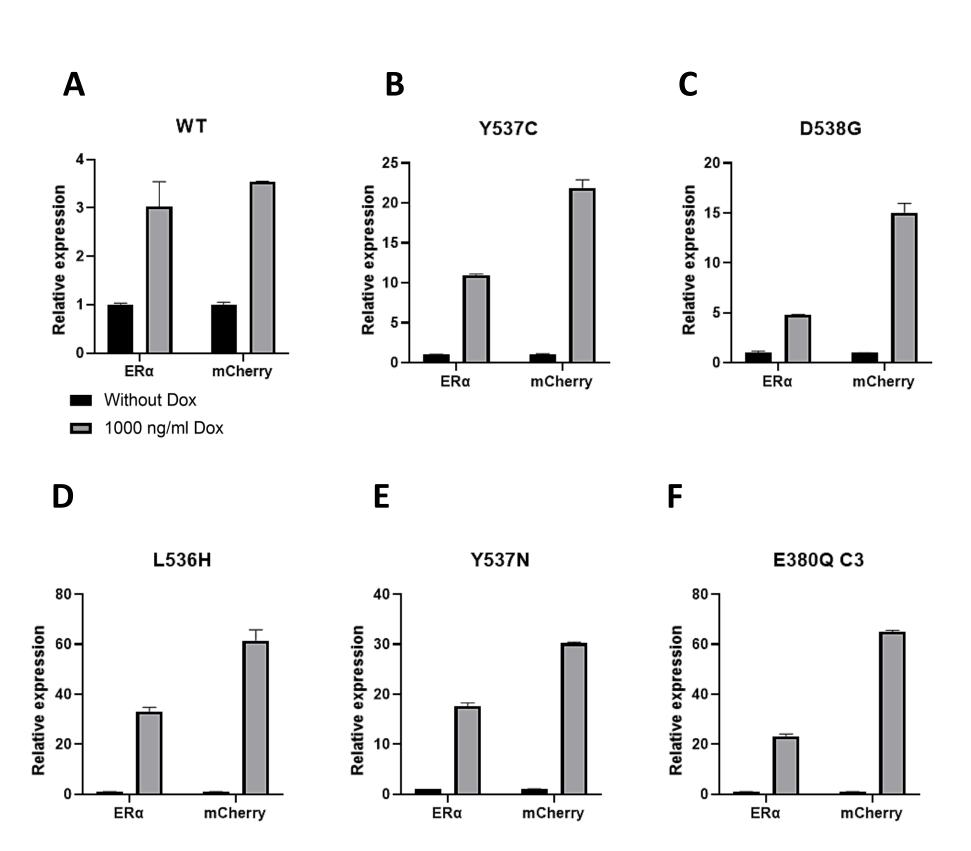


Figure 2: Study of the inducible character of the expression of the ER and mCherry variants by RT-qPCR. BT-20 cells expressing the transactivator and pTRE3G-mCherry vector containing ERα WT or its mutated variants were stimulated or not with 1000 ng/ml doxycycline (Dox) for 24 hours. Total NRAs were extracted and analyzed by RT-qPCR. The histograms corresponding to the quantification of the expression of ERα WT (A), Y537C (B), D538G (C), L536H (D), Y537N (E) and E380Q (F) and mCherry are presented and correspond to the relative expression relative to the reference genes (RPS18 and RPL32).

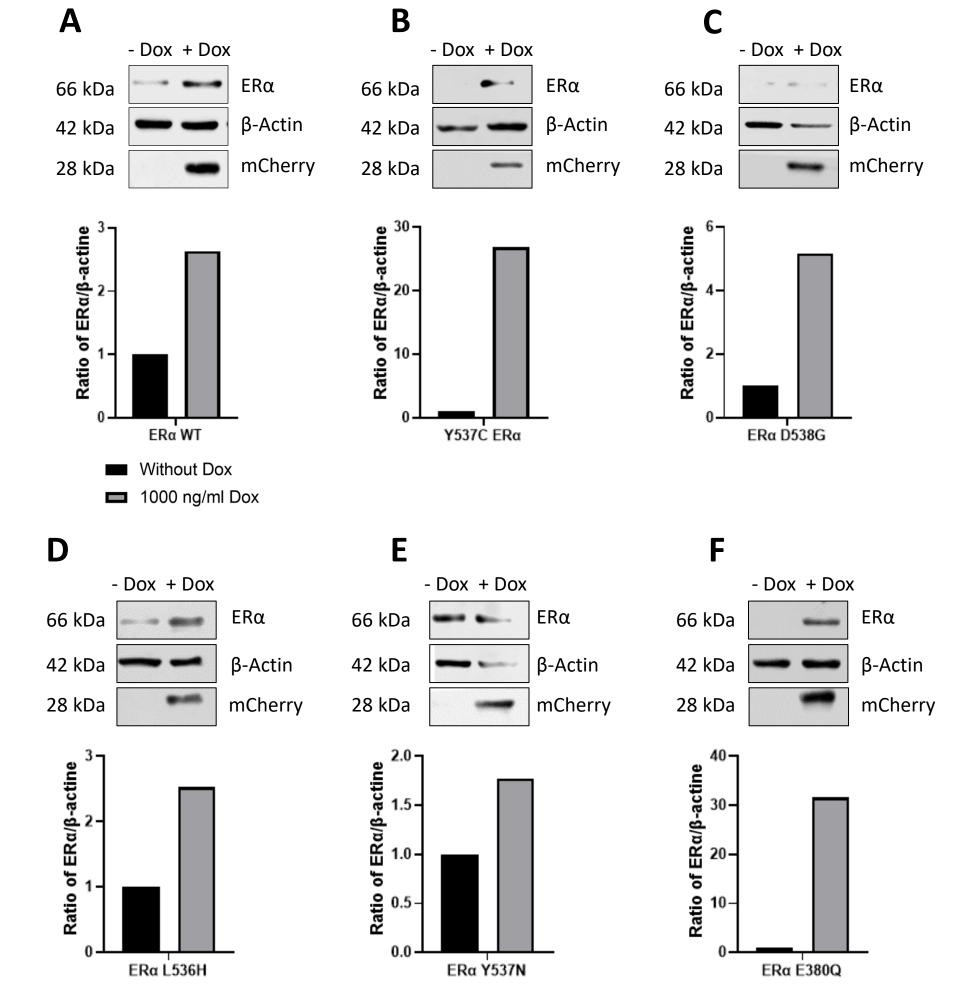


Figure 3: Study of the inducible character of the expression of the ER and mCherry variants by Western Blot. BT-20 cells expressing the transactivator and pTRE3G-mCherry vector containing ER $\alpha$  WT or its mutated variants were stimulated or not with 1000 ng/ml doxycycline (Dox) for 24 hours. The total proteins were extracted and analyzed by SDS-PAGE electrophoresis then western blotted. Western blot results as well as histograms corresponding to the relative quantification of the expression ratio ER $\alpha$ / $\beta$ -Actin WT (A), Y537C (B), D538G (C), L536H (D), Y537N (E) and E380Q (F) between cells treated or not treated with Dox are presented (n=1).

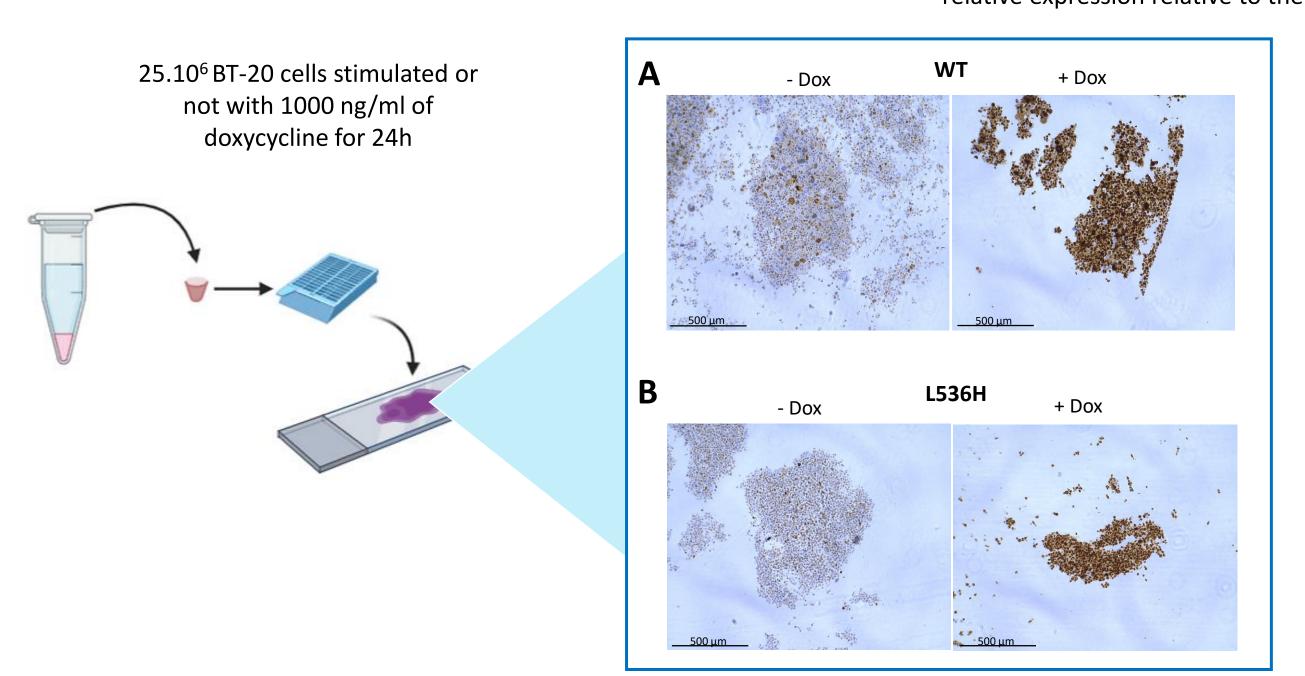


Figure 4: Study of the inducible character of the expression of the L536H variant of the ER $\alpha$  by immunohistochemistry. BT-20 cells expressing the transactivator and pTRE3G-mCherry vector containing ER $\alpha$  WT or mutated variant L536H were stimulated or not per 1000 ng/ml doxycycline (Dox) for 24 hours. The cells were then recovered and centrifuged, and the cell bases were attached with 4% paraformaldehyde for 48 hours. An anti-ER $\alpha$  immunolabeling followed by an HES staining were performed (Dako Omnis Agilent). Cells expressing the ER $\alpha$  WT (A) and L536H (B) were observed and photographed under a microscope (Evos Thermofisher FI Auto 2).

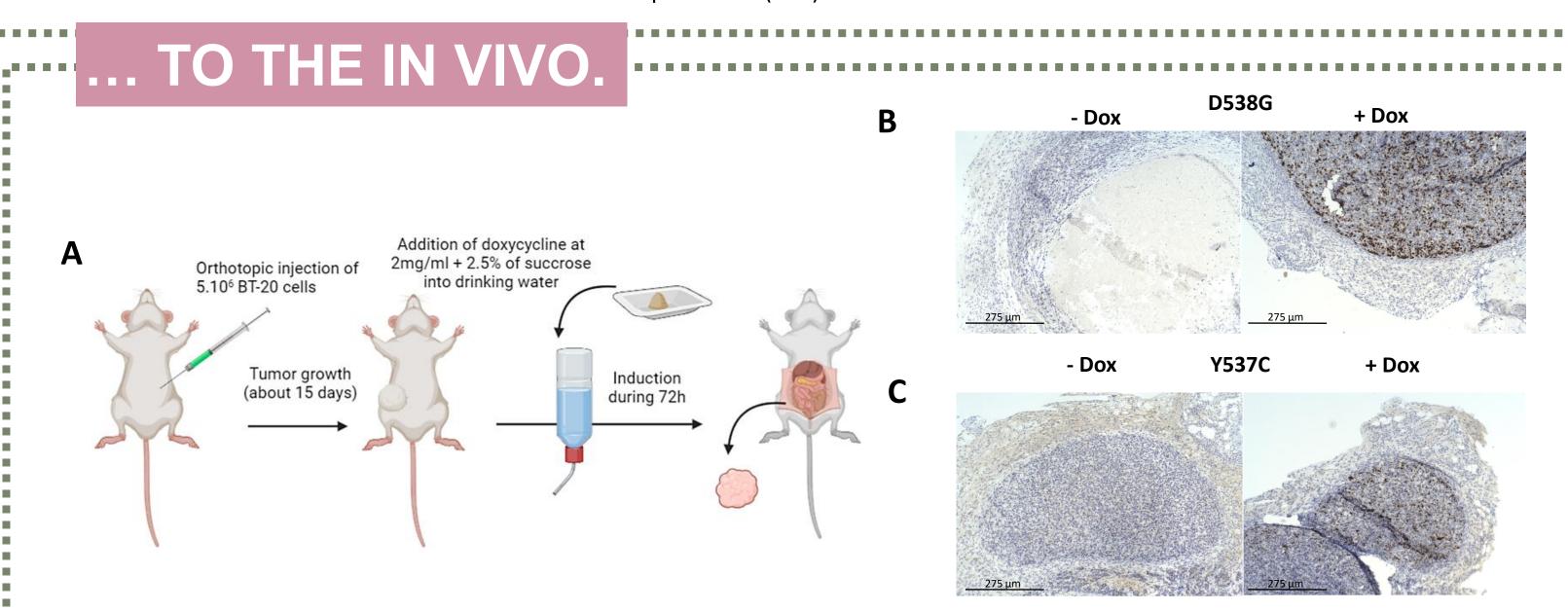


Figure 5: Study of the inducible character of the expression of ERα variants in vivo by immunohistochemistry. Five millions BT-20 cells expressing the transactivator and pTRE3G-mCherry vector containing ERα WT or its mutated variants were orthotopically injected into the SCID BALB/c (A) mouse fat pad. 15 days post injection, mice were watered with 2 mg/ml of doxycycline + 2.5% sucrose during 3 days. Then, mice were euthanized and the tumors recovered and fixed in formalin for 48 hours. An anti-ERα immunolabeling followed by an HES staining were performed (Dako Omnis Agilent). Tumors expressing ERα D538G (B) and Y537C (C) were observed and photographed under a microscope (Evos Thermofisher Fl Auto 2).

## CONCLUSION

All the results obtained allowed us to demonstrate the proper functioning of the Tet-ON system for the ERα and its studied variants. After 24 hours of stimulation at 1000 ng/ml doxycycline, we first validated the fluorescence emission of our reporter gene mCherry by fluorescence microscopy (Figure 1). Then the inducible expression of the different ERα variants were validated at the transcriptional level by RT-qPCR (Figure 2), at the protein level by western blot (Figure 3) and immunohistochemistry (Figure 4).

Finally, we tested our in vivo model for some variants, which allowed us to validate its inducibility in a orthotopical xenograft fat pad model where doxycycline (2mg/ml) was added in drinking water for 72 hours. This model will soon be used in collaboration with NancycloTEP, to study and quantify the uptake of 18FES in µPET imaging and to clarify the interest of its use in the diagnosis and follow-up of patients with hormone-dependent breast cancer.