Preclinical Explant Model of the Normal Human Breast Mimics Clinical Trial Results

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Introduction

Tamoxifen, an anti-oestrogen, is the only licensed medication in the UK for breast cancer prevention in high-risk pre-menopausal women. Tamoxifen can reduce an individual's risk of developing breast cancer by 40% following 5 years of treatment, and this reduction persists for at least 15 years. Despite this, data from the **B**iomarkers of **B**reast **C**ancer **P**revention (BBCP) trial shows that whilst there is an overall reduction in proliferation (surrogate biomarker of risk) following tamoxifen treatment a subset of women show either stable or increased proliferation in response to treatment. This highlights a requirement for new preventative therapeutics which can reduce the risk of breast cancer in pre-menopausal women who will not respond to tamoxifen

New drugs require preclinical testing, but existing prevention models are limited to in vitro studies primarily relying on human cell lines or single cell suspensions produced from primary human tissue. Both options lack the architectural complexity and microenvironmental interactions present in breast tissue. To address this limitation, we developed an organotypic ex vivo model which maintains the structural and cellular heterogeneity of normal human breast tissue, including epithelial, stromal, and immune components, for at least 14 days.

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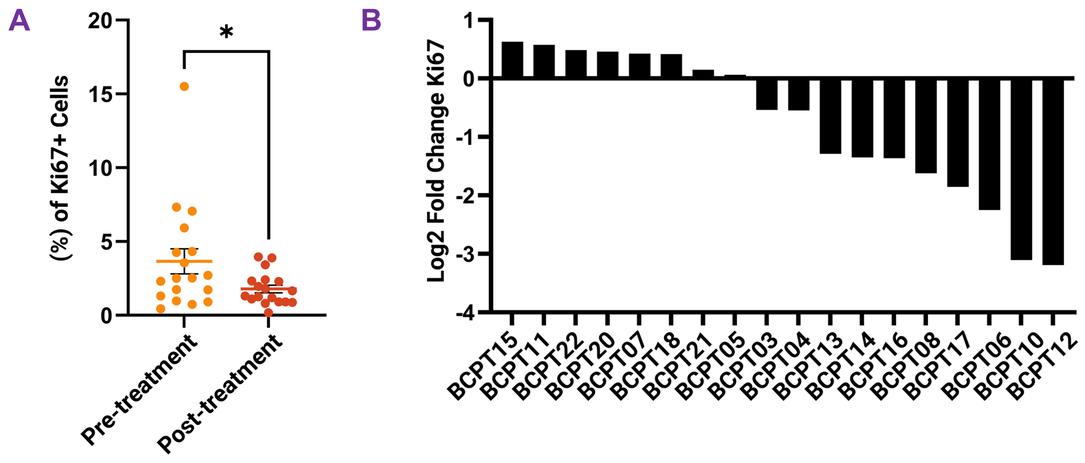
Aim

To validate an explant model of the normal human breast as a preclinical platform for the discovery of novel preventive therapeutics.

Progress to Date

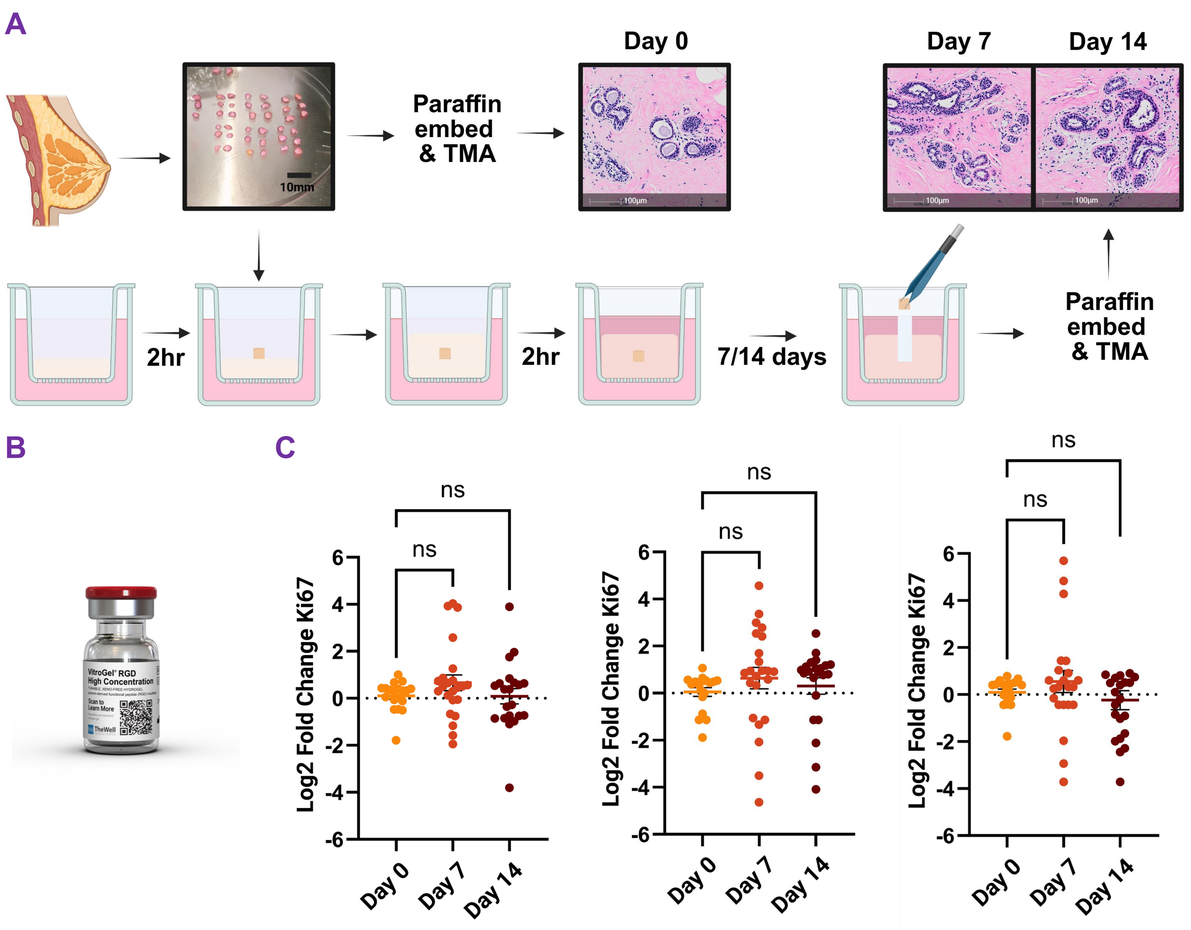
Using this model, we generated tissue arrays of normal breast tissue, obtained from risk-reduction mastectomies, cultured in the presence of tamoxifen. The effects on oestrogen receptor signalling were evaluated through immunohistochemical analysis of Ki67 (widely used as a clinical endpoint in prevention studies).

Figure 1: BBCP Trial Highlights Differential Response to Tamoxifen



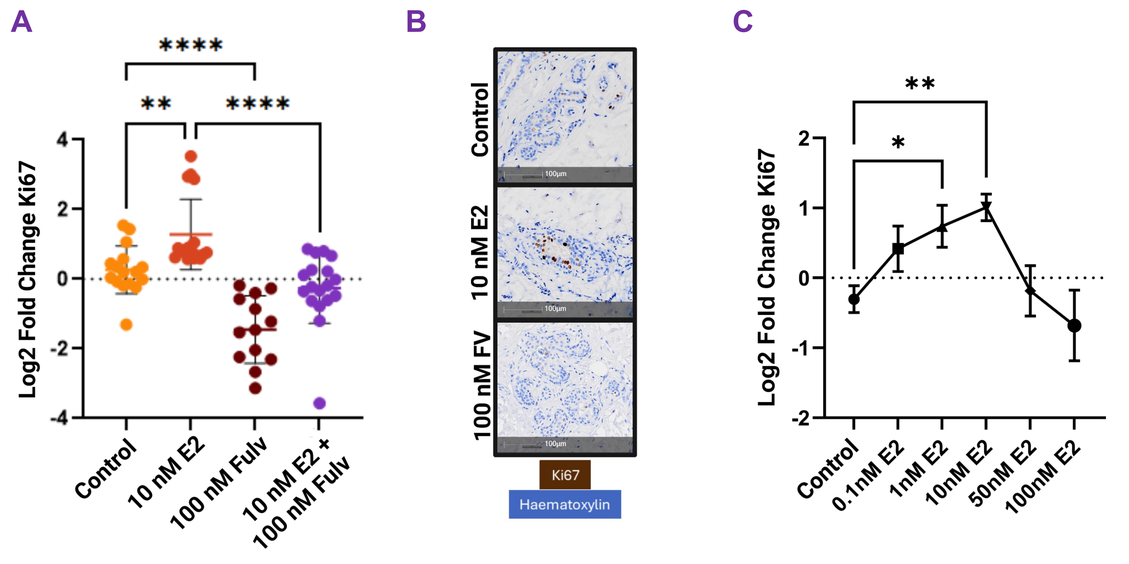
Vacuum assisted biopsies were taken from pre-menopausal women during the luteal phase of their menstrual cycle pre- and post- 3-months of tamoxifen treatment (20mg daily). Biopsies were formalin fixed, and paraffin embedded prior to immunohistochemical analysis with Ki67 (marker of proliferation). **A.** Overall decrease in proliferation across all participants following tamoxifen treatment (n=18). **B.** Differential Ki67 response seen between patients – whilst most individuals have decreased Ki67 (10/18), a subset have maintained or increased proliferation (8/18) following treatment (n=18). Data presented as either percentage of Ki67 positive cells ± SEM (A) or mean Log2 fold change (B). *P<0.05, paired t-test.

Figure 2: Normal Breast Explant model keeps tissue stable for at least 14 days



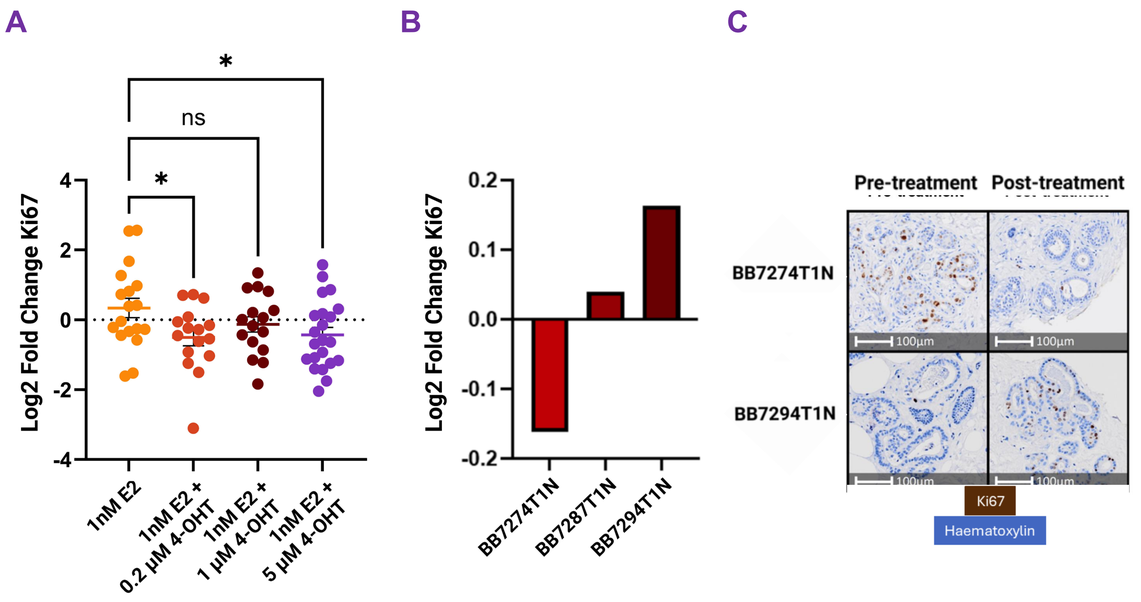
A. Fresh tissue was collected from women at increased risk of breast cancer, from the Manchester Cancer Research Centre Biobank (Biobank ethics: 18/NW/0092), and dissected into 2-4 mm chunks/ explants within 24 hours of surgery. Tissue explants were cultured within an animal-free hydrogel matrix (450 Pa, VitroGel RGD, TheWell Bioscience) for up to 14 days prior to formalin fixation and paraffin embedding (FFPE). Typical ductal/lobular structures are maintained throughout the 14 days of culture (H&E). **B.** Hydrogel used in explant model (VitroGel RGD, TheWell Bioscience) **C.** Using this method, no significant changes in proliferation (Ki67), oestrogen receptor (ER) or progesterone receptor (PR) were seen following 14 days of culture as assessed by immunohistochemistry (IHC) staining (n=5). Data presented as mean Log2 fold change ± SEM. ns = not significant, one-way ANOVA.

Figure 3: Normal Breast Explant Model Maintains Hormone Responsiveness



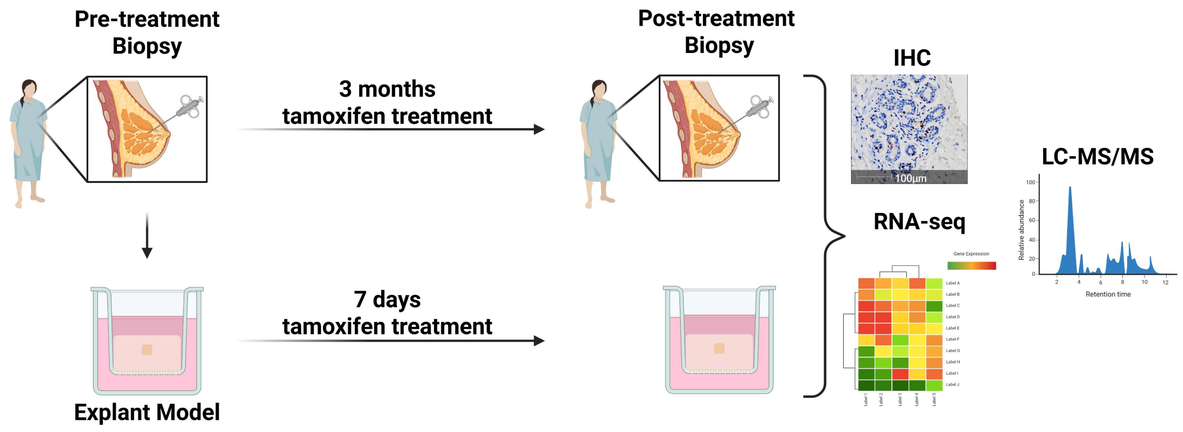
A. Treatment for 7 days with 10 nM 17β-oestradiol (E2) drives proliferation, whilst treatment with 100 nM of Fulvestrant (Fulv) inhibits proliferation (n=12) indicating responsiveness of the ER. **B.** Representative IHC images are shown. **C.** Tissue was treated for 7 days with varying concentrations of E2, revealing a bell-shaped dose-response curve previously observed in breast cancer but not described for normal breast tissue. Notably, high concentrations of E2 (>50 nM) had no effect on proliferation, while lower concentrations (1-10 nM) stimulated proliferation. This underscores the importance of selecting clinically relevant concentrations of E2 and potential xenoestrogens, to ensure that observed effects accurately reflect physiological responses. Data presented as mean Log2 fold change ± SEM. *P<0.05, **P<0.005, ****P<0.00005, one-way ANOVA.

Figure 4: Explant Model of the Normal Human Breast Mimics BBCP Trial Results



A. Similarly to the BBCP trial data (Figure 1A) the explant model shows there is an overall decrease in proliferation within the cohort when treated in the explant model with the primary active metabolite of tamoxifen, 4-hydroxytamoxifen (4-OHT), for a period of 7 days (n=4) **B.** Despite an overall reduction in Ki67, there is differential Ki67 response between individuals, with some individuals having increased Ki67 and others decreased Ki67 following treatment with 4-OHT (n=3). **C.** Representative IHC images are shown. Data presented as mean Log2 fold change ± SEM. *P<0.05, one-way ANOVA.

Figure 5: Future Work - Validating Individual Drug Response Using Matched Patient Samples



Pre-menopausal women in the luteal phase of their menstrual cycle will be biopsied pre- and post- 3-months of tamoxifen treatment as part of the BBCP trial, and response analysed using IHC, RNA-sequencing and proteomics. Using these same samples, pre-treatment biopsies will be subjected to tamoxifen (4-OHT) in the explant model for a period of seven days. Individual response to tamoxifen seen in the trial will be compared to response in the explant model to determine whether the explant model can accurately predict individual response to different therapies – this would validate the it as a preclinical platform capable of identifying novel therapeutics and personalising preventative approaches.

Conclusions

1. **Demonstrated** that the explant model can maintain normal human breast tissue including proliferation, hormone receptor expression and tissue structure over 14 days in culture.
2. **Proven** that the model is responsive to endogenous hormones and inhibitors throughout 7 days in culture
3. **Shown** that response to tamoxifen in the explant model following 7 days of treatment appears to mimic the clinical trial data with an overall decrease in proliferation despite differential participant response.

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