

# Assessing efficacy of combinational therapy of Rapamycin and Tamoxifen on estrogen receptor positive MCF7 breast cancer cells

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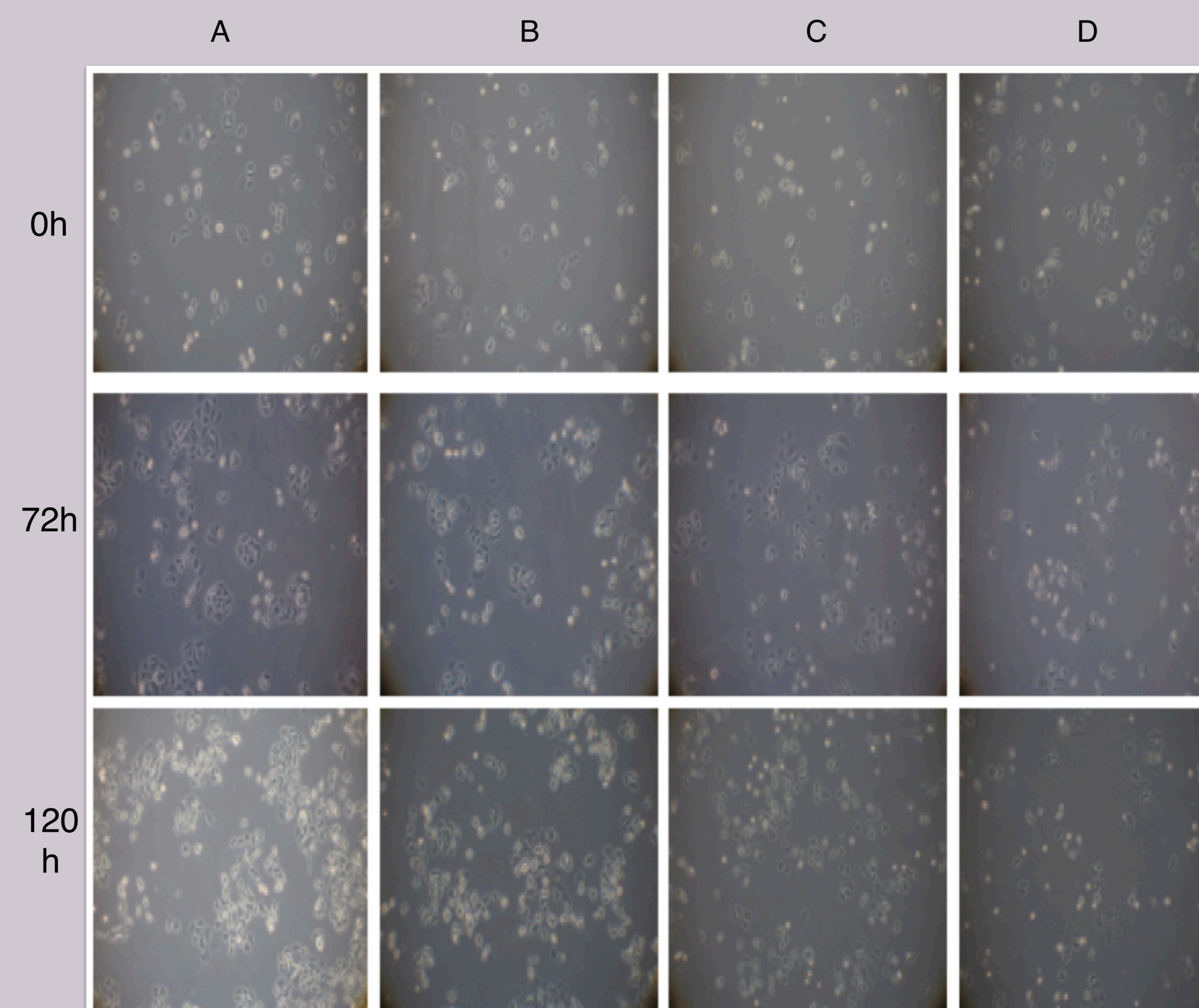
## Introduction

- Regardless of current advances in screening and therapy, breast cancer remains the second most common cause of cancer death in females.<sup>2</sup> A common and initially successful treatment for estrogen receptor positive (ER+) breast cancers is Tamoxifen. However, after long-term treatment, majority of the cases become resistant to the antiestrogen.
- To avoid the development of resistant breast cancers, an alternative method of treatment must be studied. The average clinical dose of Tamoxifen patients are given is 2  $\mu\text{mol}$ . The lower dose of Tamoxifen is proposed to increase cancer stem cell (CSC) population and lead to acquired resistance to the antiestrogen.
- Combinational therapy can increase the dose of Tamoxifen given to patients and prevent CSC population from increasing, while blocking other possible survival pathways.
- In order to study possible targeted pathways and the efficacy of the combinational therapy, a drug assay was conducted on MCF 7 cells. The assay involved varied doses of Rapamycin and Tamoxifen.

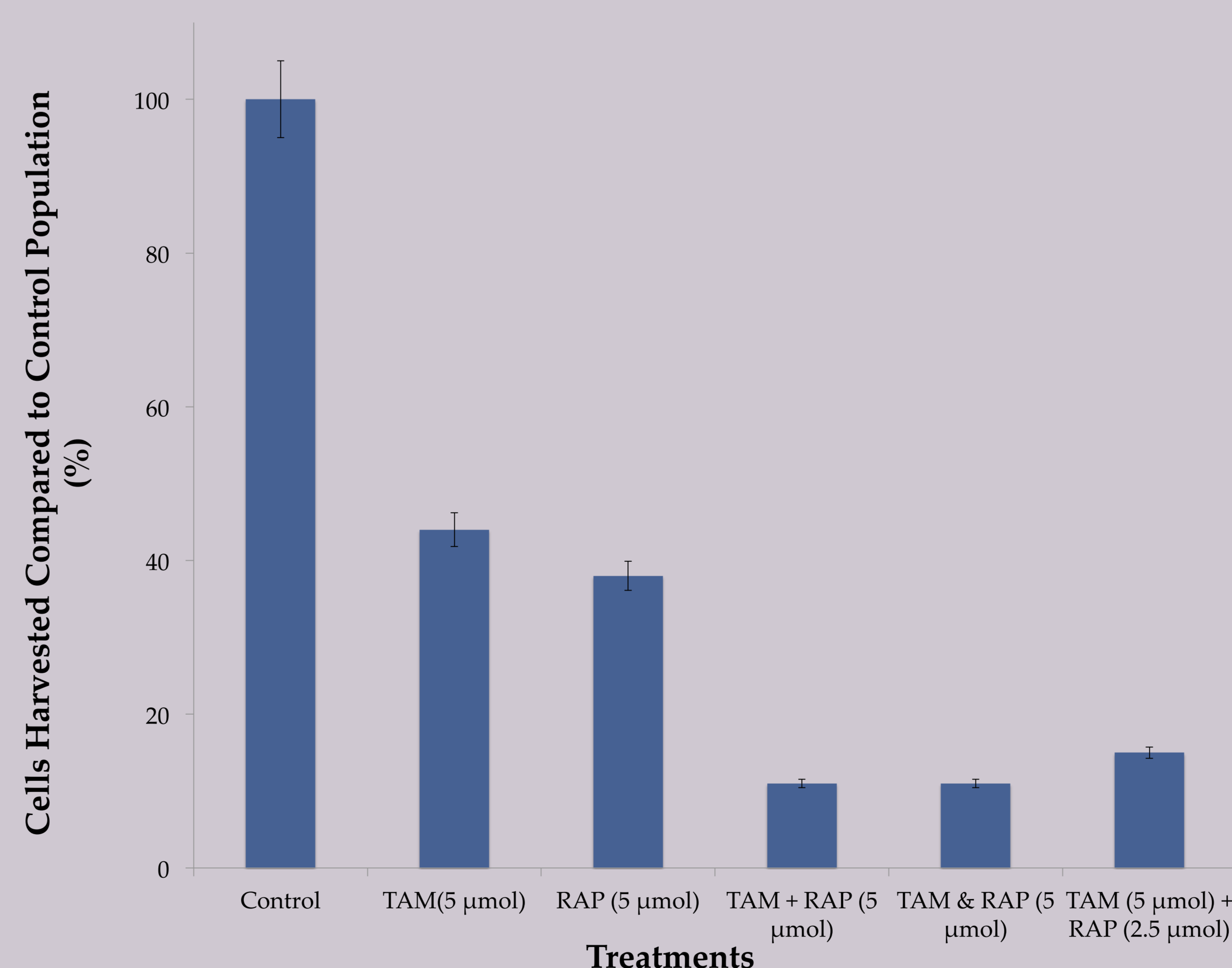
## Methodology

- 800 000 MCF 7 cells are cultured in a T75 flask with normal media (DMEM with phenol red, 5% phenol calf serum, 1% penicillin) and left in the incubator at 37°C with 5% CO<sub>2</sub>.
- The cells are split and 50 000 cells are seeded in each well containing 2 mL of media on a 6-well plate and placed in the incubator for 24h.
- Every 24h, each well is treated with its respective drug and incubated. The first well (control), is given 2  $\mu\text{L}$  of DMSO. The second well is treated with 1  $\mu\text{L}$  of Tamoxifen (5  $\mu\text{mol}$ ). The third well is treated with 1  $\mu\text{L}$  of Rapamycin (5  $\mu\text{mol}$ ). The fourth and fifth wells are treated with 1  $\mu\text{L}$  of both Tamoxifen and Rapamycin. The sixth well is treated with 1  $\mu\text{L}$  of Tamoxifen and 0.5  $\mu\text{L}$  of Rapamycin.
- After 120h (5 days) of treating, cells from each well are collected with Trypsin (0.25% Trypsin-0.52 mM EDTA) and PBS. Once the cells are harvested, 12  $\mu\text{L}$  of Trypan blue dye and 12  $\mu\text{L}$  of the cell solution are combined together and counted using a hemocytometer.

## Results

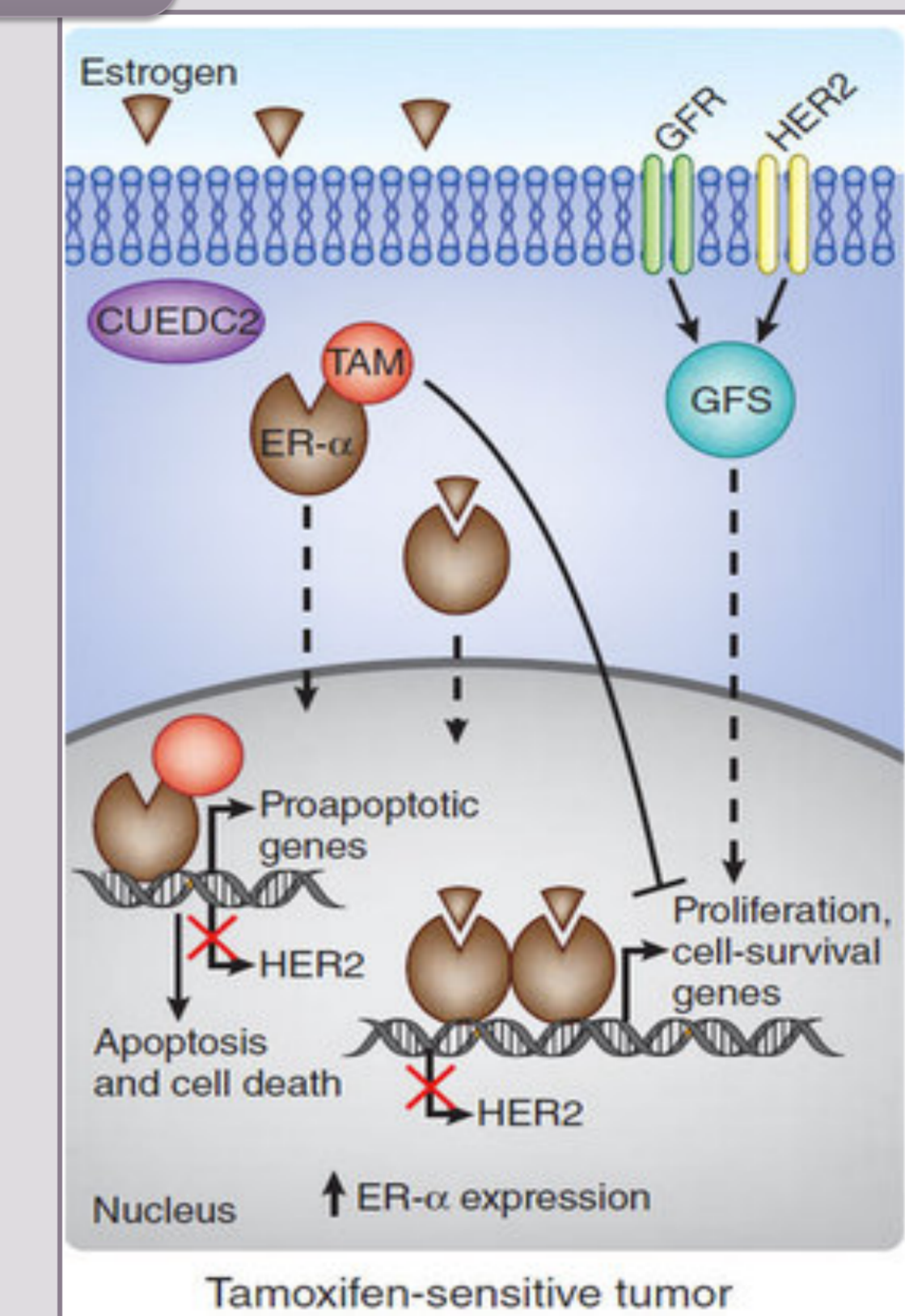


**Figure 1. MCF7 cells response to drug assay at 0h, 72h, and 120h.** Images were taken with 58mm-52mm lens at 10X magnification. The assay plate contained 6 wells, one well for each treatment. Sample images from 4 of the 6 wells; control 5  $\mu\text{mol}$  DMSO (A), Tamoxifen 5  $\mu\text{mol}$  (B), Rapamycin 5  $\mu\text{mol}$  (C), and Tamoxifen and Rapamycin 5  $\mu\text{mol}$  each (D), are shown.



**Figure 2. Percentage of cells harvested after treatments, compared to total harvested control population (170 000 cells).** After 120h of treatment, the cells were collected from each well, stained with Trypan blue, and counted with a hemocytometer. During the treatments the control well was treated with DMSO. The significantly reduced cell population observed in the combinational drug treatment of Tamoxifen (TAM) and Rapamycin (RAP) displays important implications on the efficacy of combinational therapy.

## Discussion



**Figure 3. ER- $\alpha$  expression in Tamoxifen sensitive tumour<sup>3</sup>**

- ER+ breast cancer cells need estrogen in order to grow and proliferate. Tamoxifen works as an antiestrogen by competing with 17 $\beta$ -estradiol for the estrogen receptor site (ER- $\alpha$ ) and blocks transcription of cell survival genes<sup>6</sup>.
- These long-term estrogen deprived cells often rely heavily on P13K/mTOR pathways and acquire resistance by activating this pathway<sup>4</sup>. Rapamycin inhibits mTOR, consequently halting its activities in cell cycle progression<sup>1</sup>.
- CSCs are also considered in the case of conferred resistance. Acquired properties of CSCs cells such as DNA repair ability and expression of antiapoptotic proteins contribute to drug resistance<sup>5</sup>.
- Targeting both ER and mTOR pathways through combinational therapy has effectively reduced the population of MCF 7 cells.
- Additional conclusive data can be obtained by further drug screenings using analytical tools.

## References

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## Acknowledgements

Thank you to Dr. Lisheng Wang and Andrew Sulaiman for guidance and assistance throughout the project.