Anti-Cancer Drugs Effect on Quasi-palindrome Mutations in *E. coli*

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Introduction

- Quasi-palindrome (QP) mutations are almost perfect inverted repeats of nucleotides capable of forming DNA secondary structures such as hairpins, which can block DNA replication and increase the chances of mutation (1)
- If DNA replication is blocked by secondary DNA structures, the DNA polymerase switches to the alternative DNA strands, and continues to use this as its template. This mutation is known as "Template-switching", and it results in a perfect palindrome mutation from a quasi-palindromic sequence (1,2)
- There are many known side effects of anticancer drugs, including increased intensity of DNA mutation, since these drugs work in a non-specific manner that affects all body cells.

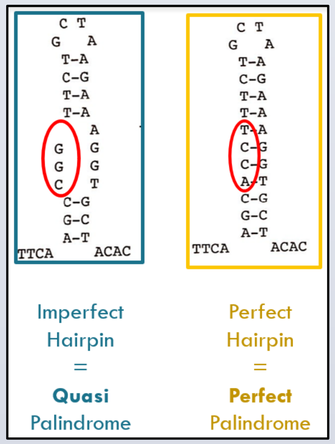

Figure 1: At imperfect hairpin sites, or Quasi palindromes, a mutation can occur creating a potentially more stable secondary structure; a perfect hairpin making into a perfect palindrome.

Objective

This study aims to evaluate the rate of Quasi-palindrome mutations in *E. coli* after exposure of selected FDA-approved chemotherapeutic drugs. This research has the potential to give us further understanding of the effect of anti-cancer drugs during DNA replication.

Materials & Methods

- Using a chromosomal reporter in *E. coli*, specific for QP mutations, the rate of template switch mutations in the LacZ gene can determined (1,2)
- A template switch mutation in the QP reporter makes a non-functional LacZ gene functional, thus reporting only QP mutants (1,2)

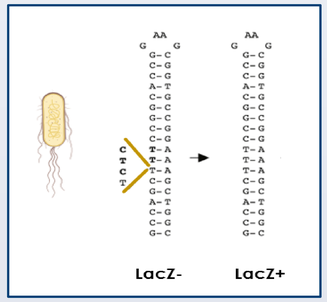

Figure 2: This reporter was created using a natural hairpin site in *E. coli* found at the LacZ gene. The chromosomal reporter will indicate an activated gene which can only happen through a template-switch mutation.

- Template switching events are analyzed and confirmed by DNA sequencing.
- In individual trials, the *E.coli* chromosomal reporter is treated with Taxol, Fulvestrant, Exemestane, Tamoxifen citrate, and Letrozole.

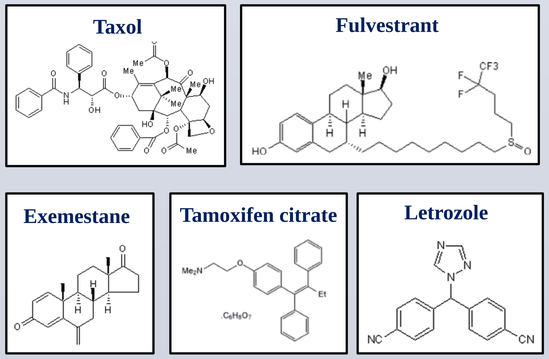

Figure 3: Molecular structure of the drugs selected for this experiment (3).

Expected Results

- We expect the rate of QP mutations to be higher after treatment of all the chemotherapy drugs we selected.
- Tamoxifen citrate and Fulvestrant can affect the rates of QP mutation because their prolonged binding to the nuclear chromatin results in impaired DNA polymerase activity in eukaryotes. Therefore, it can have a similar effect in bacterial cells if it blocks DNA polymerase (3)
- Taxol binds to tubulin and inhibits the disassembly of microtubules, thereby resulting in the inhibition of cell division in eukaryotes. It is assumed that a similar process will happen in bacteria since cell division is controlled by the tubulin homolog FtsZ (3)
- Exemestane and Letrozole are a steroidal inhibitor of aromatase (a cytochrome P450). Inhibiting enzymes within this family of enzymes may effect the process of DNA replication in bacteria as well, thus increasing QP mutation rates (3)

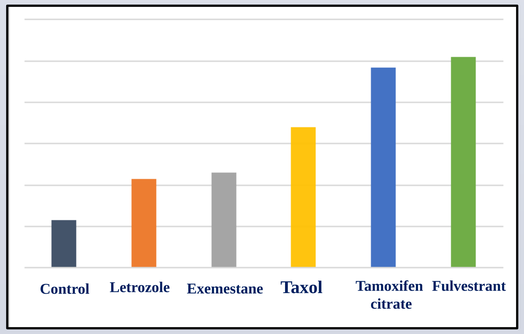

Figure 4: Predicted comparative levels of mutations after treatment with selected drugs. We predict that Tamoxifen citrate and Fulvestrant will have the highest impact on QP mutations because of their direct effect on DNA polymerase. Exemestane and Letrozole are expected to have the lowest impact of QP rates because they target enzymes within the ER away from DNA replication. Taxol should give a mutation rate in between these values.

Future Directions

- We will test these drugs to confirm our predictions for their impact in QP mutation rates.
- We will also set up experiments using these drugs in conjunction with each other since patients undergoing chemotherapy typically receive more that one drug at a time.
- We will do a screen for other drugs in the library for their ability to increase QP mutation rates.

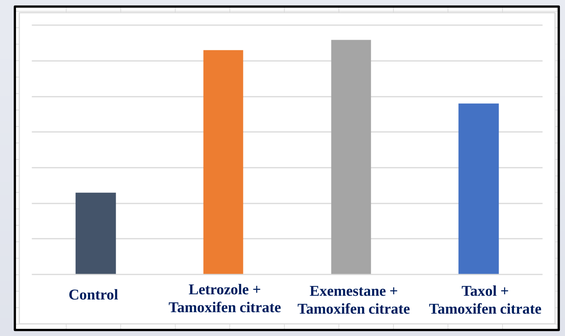

Figure 5: Predicted comparative levels of mutations after treatment with selected drugs in conjunction with others. We predict that Tamoxifen citrate and Letrozole would create a higher level of mutations compared to their individual treatment because these combinations are frequently used in the treatment of cancer patients.

References

1. Laranjo, L. T., S. J. Gross, D. M. Zeiger and S. T. Lovett, 2017 SSB recruitment of Exonuclease I aborts template-switching in Escherichia coli. DNA Repair (Amst) 57: 12-16.

2. Laranjo, L.T.; Klaric, J.A.; Lovett, S.T. Stimulation of Replication Template-Switching by DNA-Protein Crosslinks. 2018, 2018110046 (doi: 10.20944/preprints201811.0046.v1)

3. Please refer to #5932-Library of FDA-Approved Compounds "A Library of 159 'FDA-Approved' compounds – a subset of the Tocriscreen Plus Compound Collection" for a complete Certificate of Analysis

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