

Chemosensitizers: The Answer to Cancer

Noorain Shethwala & Sofia Oke

Background. Since chemotherapy was first recognized as a proven treatment for cancer, it has been subject to intense scientific scrutiny. Chemotherapy refers to the treatment of tumours through the introduction of chemicals designed to kill rapidly growing cells. Often, one major downside to chemotherapy treatments are the ever-present side effects, and the possible return of the cancer. Two of the most devastating forms of cancer, Breast cancer and Colon cancer are usually caused by defective inherited genes such as BAX, BRCA 2 and p53. The BAX gene induces apoptosis by traveling to the surface of the mitochondria of cells where it triggers the release of cytochrome c; ultimately binding to Apaf-1 protein (Apoptotic Protease Activating Factor-1). Genes such as BAX are referred to as tumour suppressors as they help control tumour growth. We have explored and will continue to explore the idea of using BAX as a chemosensitizer, which is a drug that makes tumours more sensitive to the effects of chemotherapy.

Purpose, Hypothesis. The objective of our experiment is to reduce the quantity of drug generally given to patients by making the cells more chemosensitive and prone to apoptosis through treatment with D-4EGFP adenovirus or pEGFP-BAX plasmid. With a reduction in the amount of chemotherapy drug used, there would be a reduction of side effects to chemotherapy experienced by patients (due to lower dosage). It was hypothesized that the quantity of Chemotherapy Drug administered to patients would be reduced by making cancerous cells more prone to apoptosis with the use of D-4EGFP adenovirus and pEGFP-BAX plasmid.

Procedure. The experiments were conducted with two genotypes of colon cancer cell cultures HCT 116, BAX (+/-) and BAX (-/-). Both cell lines were infected with varying concentrations of Indomethacin chemotherapy drug (250uM, 375uM and 450uM). Along with the drug, an MOI

(Multiplicity of Infection): 50 was used for the adenovirus treatments. The BAX gene, carried by a plasmid, plays a key role in inhibiting the growth of tumour cells, and thus is lacking in our two cell lines. BAX (+/-) has only one mutant allele and BAX (-/-), is lacking the functional BAX gene. An untreated control was present along with all of our cell samples of drug and drug with plasmid in an effort to compare cell viability between the different samples. The same controls were present for the adenovirus experiments. In order to keep our cancerous cells alive for the duration of our experiment, we passed the cells every two days following the cell passage protocol below.

Cell Passage Protocol:

1. From a donor flask, remove the old media and wash cells with PBS.
2. Add 1X Trypsin, pH 7.4 and incubate at 37°C for two minutes
3. Add DMEM (media with 10% FBS) directly to the cells and pipette gently.
4. Pass the cells into two T-25 flasks or 35mm Plates

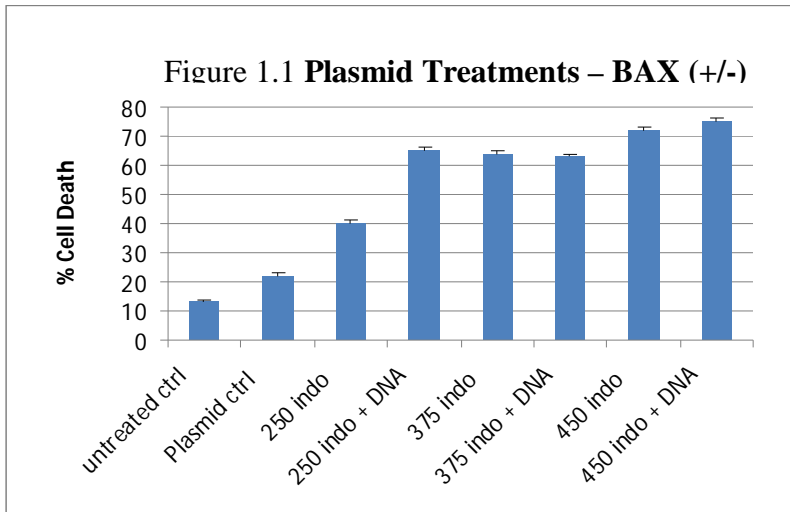
Cell structure and viability was assessed by observing cells under a microscope and by performing cell counts every 48 hours. A hemocytometer was used to assess the cell viability of our different samples. In the preliminary experiments to determine appropriate concentrations of chemotherapy drug, healthy cells were counted in comparison to dying cells for each experiment. However, the result was inconclusive and thus it was decided to use the percentage of dead cells and calculate significant differences.

Results. Preliminary results were mainly qualitative as the cell structure was assessed before performing cell counts. Initially, the impact of the three chemotherapy drugs, Indomethacin, Cyclohexamide, and Sulindac on cell structure was assessed. Indomethacin was chosen for further study as it showed distinct differences between infected (apoptotic) cells at the three concentrations and was the fastest operating. Three trials were conducted with each of our

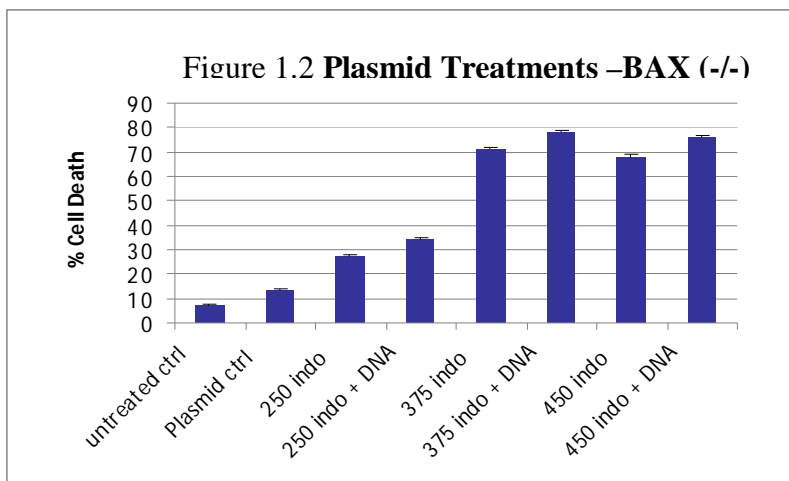
chemosensitizers and with each genotype and concentration. Two trials had technical replicates to obtain the most consistent data possible.

pEGFP- BAX Plasmid Transfection Results:
(Counted after 48 Hours)

After preliminary results from our first trial of experiments that indicated the successful transfection of BAX plasmid alongside chemotherapy drug would increase the amount of cell death, it was important to repeat our experiments three times each with technical duplicates and perform statistical analysis using Microsoft Excel ANOVA. Below are our findings for the impact of plasmid transfection into the consequent genotypes. Note: The percentage of cell death is an average of four counts.

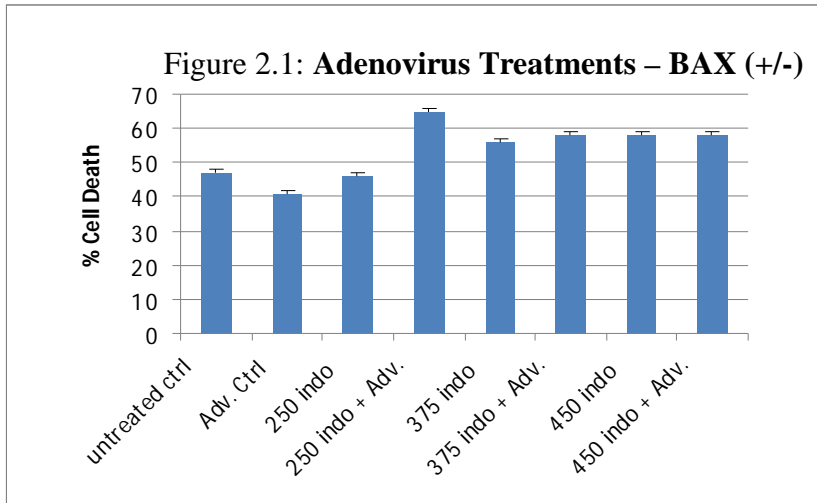


Results were analyzed at a 95% confidence and thus an Alpha value (α -value) of 0.05. After performing statistical analysis, we can conclude that the addition of a functional pEGFP BAX plasmid to a 250uM concentration of drug is **statistically significant**. Our four trials show that there is a significant 10% difference in cell death.

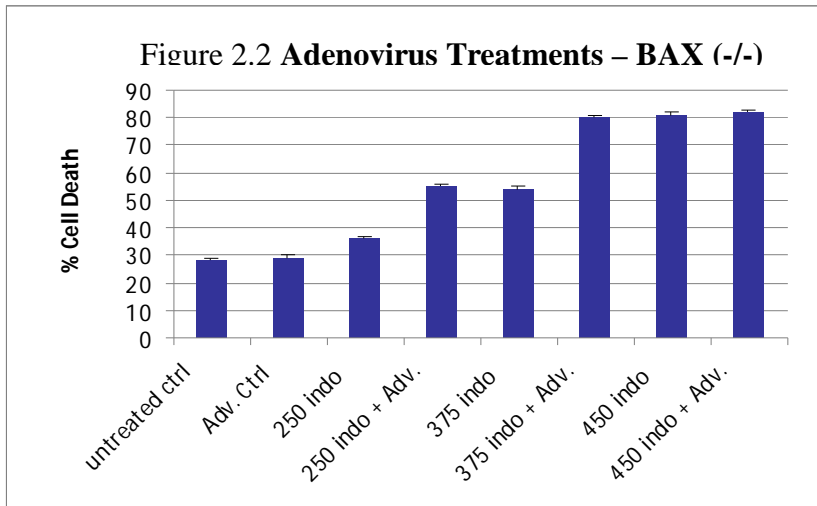


Statistical analysis has allowed us to conclude that the addition of a functional pEGFP-BAX plasmid to a 250uM concentration of drug in the BAX (-/-) genotype is **statistically significant**. Our four counts show that there is a significant **7.3%** difference in cell death.

D-4EGFP Adenovirus Infection Results:
(Counted after 48 Hours)



After performing statistical analysis, we can conclude that the addition of the fowl adenovirus to the 250uM concentration is **statistically significant** when compared to the drug alone. Our four counts show that there is a significant 18% difference in cell death.



Our results proved that there is a **significant increase** in cell death when comparing the 375uM concentration of drug alone to the addition of the fowl adenovirus to the 375uM drug concentration. The adenovirus treatment is approximately **24% more effective** in killing cells than the same concentration of drug alone.

Conclusions. After many experiments, pictures, and counts, we concluded that our hypothesis was correct. The combination of D-4EGFP adenovirus and Indomethacin drug treatment showed increased cell death compared to the drug alone in both genotypes. With respect to the BAX (+/-) genotype the addition of adenovirus to the 250uM concentration of Indomethacin proved to be statically significant whereas the 375uM concentration seemed to benefit the BAX (-/-) genotype most. Similarly, the transfection of BAX expressing plasmid showed higher levels of cell death compared to the drug alone, especially in the BAX (-/-) genotype. In the BAX (+/-) genotype we observed a significant increase in cell deaths when comparing the 250uM drug concentration, to

the 250uM drug concentration with the addition of plasmid. However, the BAX (-/-) genotype saw a significant increase of 7.3% when comparing the 250uM of drug alone to the addition of plasmid, and also saw a significant difference in cell death when comparing the 375uM concentration of drug plus plasmid to the 450uM concentration. The 375uM Indomethacin plus plasmid treatment was 10% more effective in killing the cancerous cells than the higher dosage of the drug. With this innovation we have seen indication that lower amounts of chemotherapeutic drugs can be used with either of these treatments to yield better results than higher concentrations of the drug alone. We pursued a novel approach where instead of focusing on the disease, we focused on the treatment and hope to one day provide millions of patients worldwide with this remedy.

Earlier Works. Last year only one experiment was performed that gave us leading results but nothing was repeated. This year we repeated all of our experiments four times and perfected our methods We performed the experiment over the course of four months and performed statistical analysis of our observations.

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Appendix - References & Bibliography.

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