REPURPOSING AN ANTIDEPRESSANT INTO A TREATMENT FOR LEUKAEMIA

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ABSTRACT

Repurposing drugs is the reintroduction of existing drugs into markets as treatments for conditions distinct from that which they were initially intended for. Examples of this include the use of heart medications for the treatment of erectile dysfunction, and the use of erectile dysfunction medications for the treatment of jetlag. Prozac® is an antidepressant that is widely prescribed for conditions such as obsessive-compulsive disorder and major depressive disorder. It functions by blocking the serotonin transporter, which delays the removal of serotonin, and leads to prolonged sensations of happiness and satisfaction. Chronic myeloid leukaemia (CML) is a white blood cell cancer that is currently treated using a drug called Gleevec®. Gleevec® successfully cures 12% of patients; however, 26% completely fail the treatment and 62% require lifelong Gleevec® to prevent the resurgence of the disease. This clearly demonstrates the necessity of developing a new drug that would address the 88% of patients who remain uncured of CML. It has recently been demonstrated that CML cells rely on signals from the nervous system, including signals from the serotonin transporter, to maintain survival. It was thus theorised that blocking the serotonin transporter using Prozac® should reduce the viability of the cells. The experiments performed demonstrate that Prozac® eliminates almost 60% of cancer cells and that combining it with Gleevec® further increases the proportion of eliminated cells up to 80%. This illustrates the possibility of repurposing Prozac® from an antidepressant to a treatment for CML.

INTRODUCTION

Repurposing Drugs: From Heart Medication to Viagra® to Jetlag Medication

Developing new drugs is a long and expensive process that takes 10 to 15 years, costing around £1.45 billion GBP per drug from discovery to approval (Paul *et al.,* 2010). This is partly due to the intricate safety, effectivity, and efficiency conditions enforced by governments and regulatory bodies for the approval of novel drugs (Paul *et al.,* 2010). Although such conditions have shielded the public from a myriad of useless, and sometimes harmful, new drugs, they have also severely slowed down the release of useful ones. Pharmaceutical companies and research groups have thus shifted part of their focus towards the repurposing of existing drugs.

Repurposing drugs is the process of taking a drug that has an established purpose and reintroducing it to the market for a completely new purpose (Ashburn and Thor, 2004). This is appealing because the drug has already been through the laborious approval process, and so any safety concerns will have been discovered. Thus, repurposing drugs potentially saves up to 7 years of research and studies, along with all the accompanying costs (Paul *et al.,* 2010; Ashburn and Thor, 2004).

Repurposed drugs are already widely utilised in society today, with perhaps the most familiar example being Viagra®. In the late 1980s, researchers at the pharmaceutical company Pfizer Ltd developed a drug that was intended to treat high blood pressure and chest pain (Ghofrani *et al*., 2006). The drug worked moderately well on animal models and was taken into clinical trials. When administered to humans, the male patients presented an unexpected side effect, leading Pfizer Ltd to abandon the study of that drug as a treatment for cardiovascular disease and to instead market it as a treatment for erectile dysfunction. This is how Viagra® was invented (Ghofrani *et al*., 2006).

After more detailed studies into how Viagra® works, this repurposed drug was yet again repurposed for the treatment of a new condition: jetlag. New discoveries had shed some light on the molecular details of the animal wake-sleep clock, and it was found that Viagra® directly targets part of that clock in a manner that could potentially aid in jetlag recovery (Agostino *et al.,* 2007). This prompted a research team at the University of Washington to examine the feasibility of this hypothesis. The researchers subjected hamsters to conditions that mimic a travel from New York to Paris. They found that hamsters treated with Viagra® could reset their biological clocks at least 30% faster compared to those that were not treated with Viagra® (Agostino *et al.,* 2007).

This versatility in the use of one drug demonstrates the broad potential of repurposing drugs in order to address a range of more serious conditions. The importance of this is clear in the COVID-19 pandemic, where a significant proportion of research into therapeutics has focused on repurposing drugs, especially HIV and Ebola medications, to treat COVID-19 patients (Guy *et al.,* 2020). The same approach can be applied to develop treatments for cancer, as will be discussed for chronic myeloid leukaemia (CML) in this article.

Gleevec® Revolutionised the Treatment of Chronic Myeloid Leukaemia

CML is an age-related white blood cell cancer that develops due to an acquired genetic disorder, with the average age of patients being 62 years (Houshmand *et al.,* 2019; Bjorkholm *et al.,* 2011). The disease consists of three phases: chronic phase, accelerated phase, and, finally, blast crisis. The late stages of the disease are severe, with symptoms mimicking that of different cancers combined; this led to CML's high mortality rate, where it was recorded to have an 8-year survival rate of less than 15% in the 1980's (Kantarjian *et al*., 2012).

However, in the early 2000's, a drug called Gleevec® was released. Gleevec® revolutionised the treatment of CML, transforming it from a fatal disease into a controllable condition, raising the 8-year survival rate to 87% (Kantarjian *et al*., 2012). Due to its overwhelming success, Gleevec® and its derivatives remain the treatments of choice for CML patients.

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But despite reducing the mortality rate, this drug does not cure the majority of patients. In studies on CML patients taking Gleevec® for five years, 26% of patients completely fail the treatment, 62% require lifelong treatment to prevent cancer relapse, and only 12% are cured (Holyoake and Vetrie, 2017; Gallipoli *et al.,* 2011; Lavallade *et al.,* 2008; Lucas *et al.,* 2008; O'Brien *et al.,* 2003).

It is believed that the low cure rate associated with Gleevec® is due to the drug's inability to eliminate all types of cancer cells, particularly cancer stem cells. Cancer stem cells are the foundational cells that initiate the disease, and thus the implications of their survival can be severe (Holyoake and Vetrie, 2017). If Gleevec® successfully eliminates all the cancer cells in the body except for a single cancer stem cell, then it is possible that, with time, this stem cell will reinitiate the disease and the patient will relapse. This demonstrates the need for further research to develop a new treatment to cure the remaining 88% of patients.

The Nervous System Keeps Cancer Stem Cells Alive

To develop novel therapies for cancer, it is crucial to understand the methods utilised by cancer cells to survive and reproduce. In CML, cancer stem cells are the cells of interest due to their ability to cause the patient to relapse. Their elimination is therefore key to curing a CML patient (Holyoake and Vetrie, 2017). It is thus vital to understand how those cells stay alive despite the positive results presented by Gleevec®.

CML cancer stem cells rely on two types of mechanisms for their survival. Gleevec® blocks one of those two mechanisms, which is usually sufficient for the elimination of a typical CML cancer cell. However, this is not sufficient to eliminate the stem cells (Houshmand *et al.,* 2019). Rather, the elimination of cancer stem cells requires a two-pronged approach which blocks both of their survival mechanisms. (Houshmand *et al.,* 2019).

A key component of the currently unaddressed survival mechanism is the nervous system. The nervous system utilises the serotonin and the dopamine pathways to promote the survival and reproduction of the cancer stem cells (Cosentino *et al.,* 2015; Spiegel *et al.,* 2007). This discovery provided a range of possible targets that could be exploited for therapeutic purposes. It is also an ideal setup for employing the concept of repurposing drugs, due to the wide array of drugs currently in the market that target the nervous system.

A research team at the Wolfson Wohl Cancer Research Centre set out to examine the potential of using such drugs to exploit this survival mechanism and to eliminate cancer stem cells. A number of antidepressants and anti-schizophrenics are currently being tested for this purpose. This article examines the possibility of using the antidepressant Prozac® to block the cancer stem cell survival mechanism leading to their elimination. The results presented are a step forward in the development of a therapeutic method that could be employed for the treatment of CML.

METHODS

The experiments presented in this article were performed in the Vetrie Laboratory at the Wolfson Wohl Cancer Research Centre in Glasgow, UK, between September of 2019 and January of 2020.

Culturing the Cancer Cells

The experiments were performed using cells derived from the cancer cells of a 73-year-old CML patient in blast crisis. All the cells were provided with the necessary nutrients and incubated under the appropriate conditions of 5% CO_2 at 37℃. The cells were treated with a low, medium, or high dose of Prozac®, either singly or in combination with Gleevec®. This was done alongside a sample of cells, called the "no drug control", which was not treated with any drug and was used as a reference to determine the extent of the impact the drugs have on the other samples. The cells were grown for six days and were maintained at an appropriate concentration to prevent their overcrowding. Samples were then extracted at specific timepoints for the measures of interest to be determined.

Counting the Cancer Cells

The dimensions of the cancer cells were inputted into the CASY® cell counter, and the machine was then used to determine the number of cancer cells in each of the studied conditions. For figure 1, the no drug control was assumed to attain the maximum possible number of cancer cells (100%). From that, the respective number of cancer cells from each of the studied conditions was determined and plotted on the graph.

Measuring the Proportion of Eliminated Cancer Cells

An apoptosis assay, which is a well-established method for determining the number of eliminated cells (Oancea *et al*., 2006), was used in this experiment. The assay was carried out on a FACSVerse™ machine, which employs the flow cytometry technique to count the number of cells of interest. Using this approach, the proportions of dead cells on days one, two, three and six following a Prozac® treatment were determined, and the results are presented in figure 2. Similarly, the proportions of dead cells following a combinatorial treatment of Prozac® and Gleevec® for two days were determined, and the results are presented in figure 3.

RESULTS

Prozac® Reduces the Number of Cancer Cells

For an initial check of the legitimacy of the presented hypothesis, the impact of Prozac® on the number of cancer cells was studied (figure 1). Cells were treated with Prozac® for six days and their numbers were appropriately monitored. The no drug control was used as a reference to determine the impact Prozac® has on the number of cancer cells.

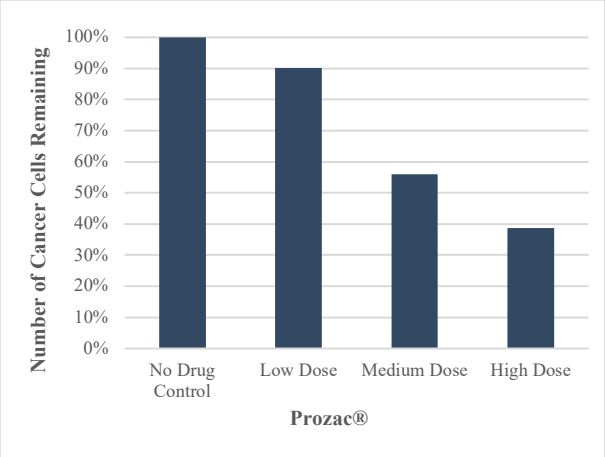

Figure 1: Proportional number of CML cancer cells remaining after treatment with Prozac®

The cells were treated with a low, medium, or high dose of Prozac® for six days. The "no drug control" is a sample of cells that was not treated with the drug.

The low dose of Prozac® reduced the number of cancer cells by 10% compared to the control. The medium and high doses of Prozac® further reduced their number by 44% and 62%, respectively. This indicates that Prozac® does reduce the number of cancer cells after six days of treatment, therefore providing solid experimental basis for continued research into this hypothesis.

Prozac® Eliminates Cancer Cells

The recorded reduction in the number of cancer cells following the Prozac® treatment for six days could either be due to the inhibition of the reproduction of the cancer cells or to their elimination. To determine if the Prozac® treatment was killing the cancer cells, an apoptosis assay was performed to measure the proportion of cells that have died after one, two, three, or six days of treatment with three doses of the drug, alongside a no drug control (figure 2).

On day one (figure 2A), the four studied conditions were found to have a similar proportion of dead cells, ranging between 45.2% and 46.4%. A clear difference amongst the conditions started to be observed on day two (figure 2B), whereby the higher doses of the drug produce a higher proportion of dead cancer cells. The no drug control had 12% dead cells, and this increases up to 18.2% and 25.8% under the medium and high doses of Prozac®, respectively. The low dose of the drug continues to record a similar proportion of eliminated cells as that of the control, where it was observed to be 12.9%.

On days three and six, the impact observed on day two becomes mostly limited to the high dose of Prozac®. On day three (figure 2C), the control was recorded to have 8.6% dead cells. The proportion of dead cells only conservatively increases to 10.9% under the medium dose of the drug, but increases more significantly up to 15.4% with the high dose. Similarly, on day six (figure 2D), only the high dose of Prozac® observed a recognisable increase in the proportion of eliminated cells, where it was recorded at 11.3%, compared to 4.8% for the no drug control. This experiment demonstrates that Prozac® does lead to the elimination of the cancer cells.

Combinatorial Treatments of Prozac® and Gleevec® Eliminate a Higher Proportion of Cancer Cells

It was then necessary to determine the best approach for the elimination of the cancer cells, and so the impact of a combinatorial treatment of Prozac® and Gleevec® for two days on the proportion of eliminated cancer cells was studied (figure 3). From the results presented in figure 2, it was clear that the

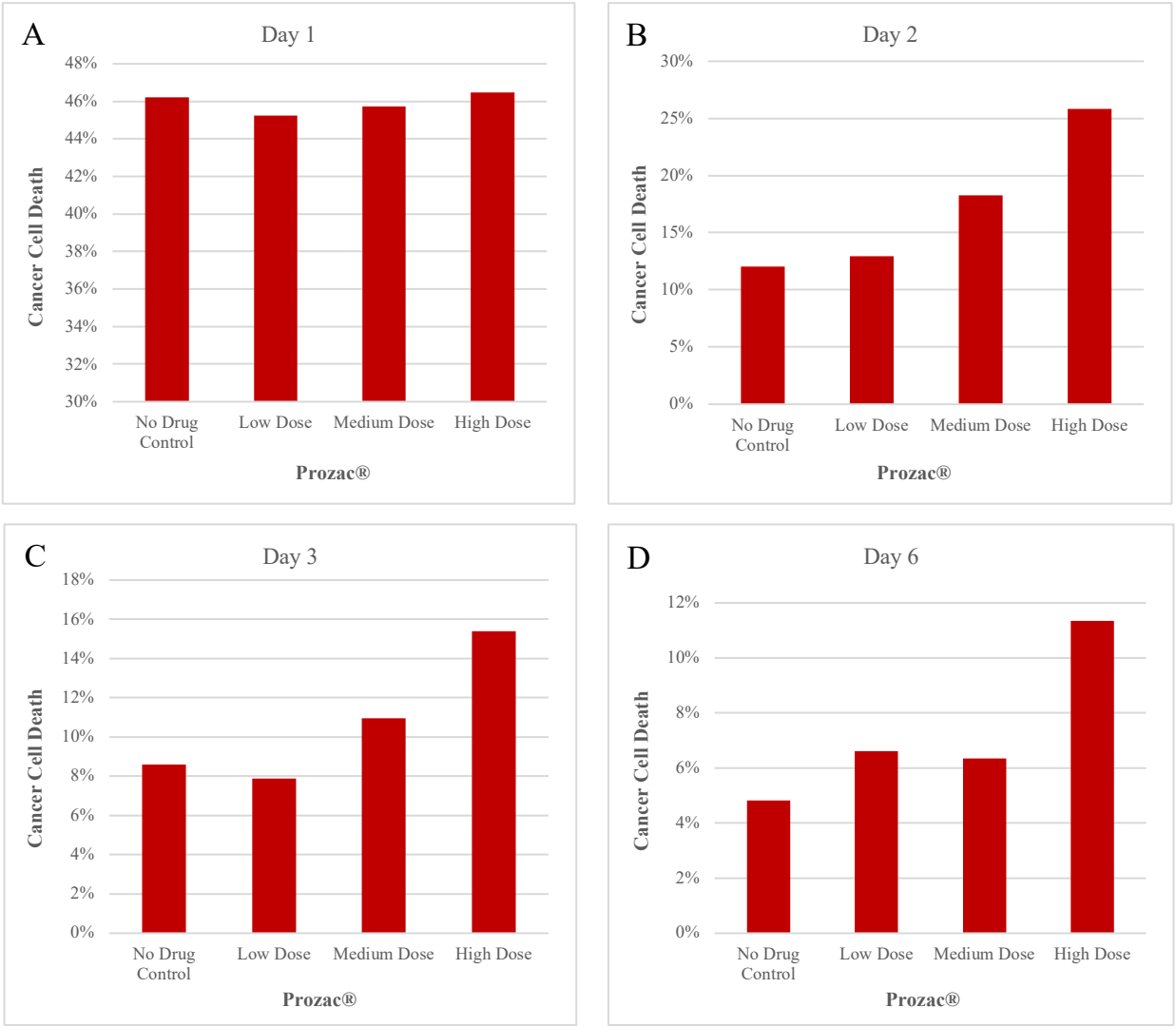

Figure 2: Proportions of eliminated CML cancer cells after one, two, three, or six days of a Prozac® treatment

The cells were treated with a low, medium, or high dose of Prozac®. The "no drug control" is a sample of cells that was not treated with the drug. (A), (B), (C), and (D) present bar graphs showing the proportions of eliminated cells on days one, two, three, and six, respectively.

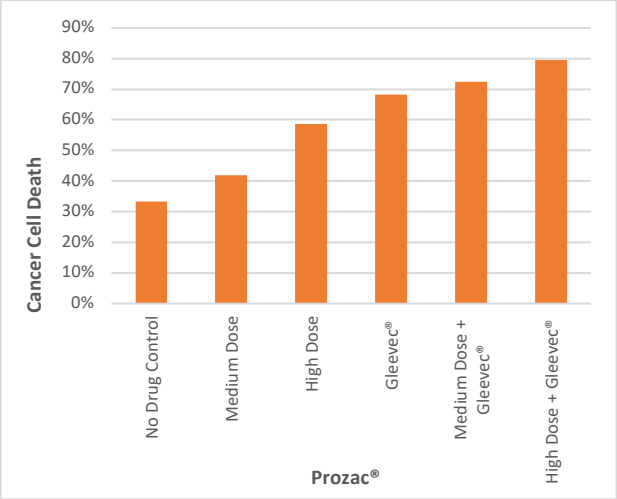

Figure 3: Proportions of eliminated CML cancer cells after two days of combinatorial treatments

The cells were treated with a medium or high dose of Prozac®, either singly or in combination with Gleevec®. The "Gleevec®" sample refers to cells that have only been treated with Gleevec®. The "no drug control" is a sample of cells that was not treated with any drug.

medium and high doses of Prozac® were the most effective at eliminating the cells, and so those two doses were studied either singly or in combination with Gleevec®. This experiment was run alongside a sample that was only treated with Gleevec® to determine how beneficial the addition of Prozac® would be. A no drug control was also studied.

The control was recorded to have 33.3% dead cells. As was shown in figure 2, both the medium and high doses of Prozac® record a higher proportion of dead cells than the control, where it was observed at 41.8% and 58.6%, respectively.

When cells were treated with Gleevec®, the proportion of eliminated cells was found to be 68.3%. This is further increased when the cells were treated with a combination of Gleevec® and a medium or high dose of Prozac®, where it was then observed at 72.5% and 79.6%, respectively. This indicates that a combinatorial treatment of Gleevec® and Prozac® provides a more effective approach for the elimination of the cancer cells.

DISCUSSION

After obtaining these results, there was strong evidence to support that Prozac® can be used to reduce the number of CML cancer cells. This is sufficient to trigger further studies into the drug; however, it is important that we first discuss how this reduction is attained. The observed reduction could either be due to the elimination of the cells or due to the disruption of their reproduction. Although there is currently no published research examining the latter, our results do allow us to make a strong prediction. The low dose of Prozac® reduces the number of cancer cells but does not lead to their elimination. Therefore, a likely explanation for this reduction is that the drug has disrupted the reproduction of the cancer cells. It is vital, however, that further studies are performed to confirm this.

Our study does, however, provide clear evidence for the former: the elimination of the cancer cells. Prozac® begins to kill the cells two days after being administered. As would be expected, higher doses of the drug eliminate the cells more successfully, with the highest dose being the most efficient. Additionally, only the high dose of Prozac® continues to eliminate the cancer cells up until the termination of the experiment on the sixth day. Those results provide an important indication as to how further studies should be carried out. We have clearly demonstrated that the low dose of the drug does not kill the cells, therefore shifting the focus of future research solely towards higher doses of Prozac®. Furthermore, the difference in the longevity of the effectiveness of the medium and high doses is of significance. It forms the foundation for studies looking into the most appropriate frequency and dispersion for the delivery of the drug that would provide the most preferable outcome.

We have thus far demonstrated the potential for the use of Prozac® for the elimination of the CML cancer cells. Combinatorial studies, where multiple drugs, in this case, Prozac® and Gleevec®, are combined, were a natural second step to determine if Prozac® can amplify the effects of Gleevec®. Combinatorial treatments are a regularly used strategy to produce the best outcome when treating difficult and severe diseases, such as cancer. However, for the combination to be employed, it has to be more effective than the standard treatment alone. Our results clearly demonstrate this. Treating cells with a combination of Prozac® and Gleevec® eliminates over 10% more cells than under Gleevec® alone. Prozac® thus promotes the effects of Gleevec®, presenting this drug combination as a strong contender for the treatment of CML.

Future Work

Because Gleevec® is the standard CML treatment, any combination that improves its effects would be useful. However, the most preferable outcome would be for the combination to eliminate all the cancer stem cells, which would reduce or completely remove the risk of relapse. Although this study confirms that the combination is more effective at eliminating a typical CML cancer cell than Gleevec® alone, we were unable to examine the impact of this combination specifically on cancer stem cells due to time constraints. It is important that this is studied to determine if Prozac® could not only be utilised as a promoter of the effects of Gleevec® but also as a method to achieve the primary goal of the elimination of cancer stem cells.

It is also important to determine the best approach for delivery of Prozac®. The first aspect of this would be determining the most preferable dose to attain the intended outcomes. Combinatorial treatments should be studied again, as was done in this article, but using combinations of different doses of both Prozac® and Gleevec®. In addition, the order of delivery must be examined. In this study, the two drugs were introduced simultaneously. The experiments should be repeated but with variations in the order of delivery. This could be done by first treating the cells with Prozac® and then, after a period of time, treating them with Gleevec®, and, conversely, starting with Gleevec® followed by Prozac®. The results of these experiments would form the foundation for how future trials could be performed.

The experiments were performed using cells derived from a single CML patient in blast crisis. Although these cells are the widely accepted cell model for CML research (Pussant *et al*., 2012), it is vital that future experiments are repeated on cells obtained directly from multiple CML patients. In doing so, the results presented in this study would be confirmed, and research into the use of Prozac® as a treatment for CML could progress into the next stage: animal trials.

Finally, it is important to realise that Prozac® may not be the only drug that could produce these effects. The work currently being performed at the Wolfson Wohl Cancer Research Centre examining the use of anti-schizophrenics and other antidepressants for the treatment of CML is key to determining the uniqueness of the results of the Prozac® treatment. In addition, if a number of drugs were found to produce similar results, then the team could identify the most beneficial, which would then be taken forward for further studies. Such a decision

would not only be based on the effectiveness of the drug, but also on the impact it would have if administered to a cancer patient. This is particularly important to consider when repurposing drugs, because the usual function of that drug itself becomes a side effect.

CONCLUSION

Repurposing drugs has become an established tool utilised by research groups and pharmaceutical companies for the faster and cheaper production of therapeutics for the treatment of conditions and illnesses varying greatly in their severity. This study functions as a proof of concept for the repurposing of the antidepressant Prozac® into a treatment for CML. Treating the CML cancer cells with the antidepressant led to their elimination – an outcome that is greatly amplified under a combinatorial treatment with Gleevec®. This article thus positions Prozac® as a candidate worthy of further research to determine its full capacity as a treatment for CML.

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