



Seeds4Hope

A program of the Windsor Essex County Cancer Centre Foundation



2011 SEEDS4HOPE GRANT RECIPIENT

Dr. James Green

“Synthesis and Evaluation of New Anticancer Alcolcolchinoids”

SUMMARY OF RESEARCH PROJECT

The purpose of the proposed research is to develop new methods to prepare a colchicine derivative (an alcolcolchicine) that has shown, in preliminary work, the ability to kill cancer cells without damaging normal cells, and to use these methods to prepare compounds closely analogous to the first derivative. The methods are intended to develop a more economical synthesis, and to generate less in the way of waste.

The novel colchicine derivatives will be evaluated for their anticancer activity in a variety of cancer cells including pancreatic and colon cancers, and for their toxicity to non-cancerous cells. We will also investigate the mechanism of cell death caused by these compounds. The promising compounds will finally be tested for their safety and anti-cancer activity in human tumors transplanted in immuno-compromised mice as well as in patient-derived blood cancer samples.

HOW THIS RESEARCH HELPS ADVANCE QUALITY CANCER CARE IN OUR COMMUNITY

Results obtained from this research project would present a new window of opportunity for the treatment of some of the most aggressive and chemo-resistant cancers by the design and synthesis of non-toxic derivatives of a toxic but otherwise effective compound. The biochemical knowledge of how these analogues selectively target cancer cells will transform the understanding of how natural compounds and their analogues can be used as chemotherapeutics; opening a new window of opportunity to treat cancer, both aggressive and non aggressive forms with safer, non-toxic derivatives of colchicine.



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PROGRESS REPORT - YEAR ONE

A: Synthesis of New Anticancer Alcolchicinoids:

Our progress in the synthetic aspects of developing catalytic routes to alcolchicinoids has been extensive. During the last year we have synthesized two potential anticancer alcolchicinoids from commercially available substrate materials, first **GREEN1**, followed by **GREEN2** (both named by a colleague). During development the synthesis of these two alcolchicinoids was shorted from thirteen to eight steps. Some optimization remains to be done.

B: Efficacy of Colchicine Analogues Against Cancer Cells:

To assess the anticancer efficacy of alcolchicine derivatives, **GREEN1** and **GREEN2** were tested against various cancer cell lines (Pancreatic, Colon cancer and glioblastoma cells for **GREEN1** and Pancreatic cancer cells for **GREEN2**), at increasing time points and concentrations. The induction of cell death was observed using specific characteristics of autophagy (Programmed Cell Death, type I) and apoptosis (Programmed Cell Death, type II). The results of these studies indicate that both derivatives of colchicine effectively target cancer cells to induce both forms of programmed cell death (PCD) in a dose and time dependent manner.

To further characterize the anticancer efficacy of colchicines analogues, these compounds were screened against non-cancerous cells, including Normal Human and Fetal Fibroblasts (NHF and NFF). Results indicate that **GREEN1** did not induce the distinct pro-death autophagy that was observed in cancer cells, however, **GREEN2** was not as selective, as it led to the induction of apoptosis in both the cancer cells and the non-cancer NFF cells, suggesting that **GREEN1** represents a potentially safer alternative to colchicine and other available analogues in its ability to selectively target cancer cells.

The next step in screening looked at the efficacy of **GREEN1** against peripheral blood mononuclear cells (PBMCs) obtained from newly diagnosed leukemia patients, in parallel with those obtained from healthy volunteers. This step is important as it highlights the efficacy of colchicine analogues against actual patient samples. Since the compound cannot be provided to patients at this stage, this is the obvious, logical step before clinical studies. Following isolation of PBMCs from the blood of leukemia patients and healthy volunteers, the cells were treated with **GREEN1**, stained with propidium iodide (PI) to indicate cell death and the results were obtained on a TALI image based cytometer. The results indicate that **GREEN1** efficiently increased the percentage of PI positive cells in the patient blood cell sample, with no effect on those cells obtained from healthy volunteers, confirming the results obtained in commercially available cancer and non-cancerous cells and indicating the selectivity and efficacy of **GREEN1** against various cancer cells.

C: Mechanism of Colchicine Derivatives Against Cancer Cells:

It was observed that both colchicine derivatives had unique targets, as they induced different forms of programmed cell death (PCD) in cancer cells. Using methods and controls that allow us to characterize the mechanism of PCD induction, we determined that **GREEN1** likely targets the mitochondria directly, whereas **GREEN2** appears to target tubulin polymerization. These results confirm that **GREEN1** and **GREEN2** have distinct biochemical mechanisms, and suggest that a simple change in the structures of these colchicines analogues leads to very different mechanisms.



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PROGRESS REPORT *cont'd*

D: Tolerability and Efficacy of Colchicine Derivatives in Xenograft models of Cancer:

To assess the efficacy of **GREEN1** in *in vivo* models, immunocompromised CD-1nu/nu mice were injected subcutaneously with 2×10^6 cells/mouse colon cancer cells (HT-29). Once the tumors were established, the mice were divided into two groups: one group (control) received vehicle injections (5% DMSO in PBS), the other group (experimental) was treated 3 times per week for a total of 41 days and 15 treatments with 10mg/kg/ml **GREEN1**, a dose that we determined was preliminarily well tolerated in mice. The tumor sizes were measured throughout the experiment and the tumor volumes were calculated. Our preliminary results show that three of the mice in the **GREEN1** treatment group had tumours smaller than 1500 mm^3 , while three of the mice in the control group had tumours that were larger than 1500 mm^3 . This suggests that **GREEN1** may have suppressed the growth of colon cancer tumors in the immunocompromised CD-1nu/nu mice. This experiment has to be repeated and confirmed in xenograft models of colon cancer and possibly other forms of cancer as well.



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PROGRESS REPORT - YEAR TWO (Final Report)

Our group, in conjunction with the group of Prof. Siyaram Pandey, have developed a novel, significantly shorter, greener, and less expensive method for the synthesis of anticancer allocolchicinoid compounds based on catalytic reactions. We have synthesized and screened anticancer activities of several compounds, including **GREEN1**, and **GREEN2** = NSC 51046, **GREEN3** and **GREEN4**. In all cases except **GREEN2**, the structures are unique isomers of the more conventionally established allocolchicine compounds, having very similar structures but with different locations of the atoms in the chemical compounds. The compound **GREEN2** is known to be active against a number of types of cancer cell lines and is less toxic than colchicine itself, but kills normal human fibroblasts and is known to still have cardiotoxicity. However, we have discovered that **GREEN1** has significant toxicity selectively to pancreatic cancer cells and leukemia (E6-1) cells, without any toxic effect to normal human fibroblasts and is tolerated in mouse animal models. This suggests that the selectivity of these isomeric allocolchicines (i.e., **GREEN1**), the ability to target cancer cells over healthy cells, is much higher than the known conventional compounds.

In addition, the mechanism of cancer cell attack by the conventional allocolchicines is well established, namely by apoptosis. Conversely, **GREEN1** (representing the isomeric allocolchicines), attacks the cancer cell lines by a completely separate mechanism, pro-death autophagy. Currently, **GREEN1** is being tested in animal models of thyroid cancer in our collaborator's laboratory at the Ontario Cancer Institute.