Seeds4Hope

A program of the Windsor Essex County Cancer Centre Foundation

2010 Seeds4Hope Grant Recipient

Dr. Siyaram Pandey

"Preclinical evaluation of anti-cancer activity and mechanism of apoptosis induction by Dandelion root extract (DRE) in in vitro, in vivo and ex-vivo models of leukemia"

SUMMARY OF RESEARCH PROJECT

Despite aggressive research efforts to find selective anti-cancer chemotherapeutics, we are still far from such agents and are still limited to the toxic treatment options including drugs and radiation therapy that cause indiscriminate killing of cancerous as well as normal healthy cells. Damage to normal, non-cancerous cells causes harsh side effects and mutations to DNA that can increase the risk of these cells becoming cancerous.

Natural products have been shown to play a prevailing role in drug development with 50% of all anti-cancer drugs approved between 1941 and 2006 being either natural products or obtained from natural sources. Within the vast repertoire of Mother Nature, there may be compounds that specifically target and kill cancer cells. Dandelions are one of the most common and recognizable weeds found in almost every part of the world. Extracts from the roots from Dandelions have been used for several decades in traditional medicine, yet very little is known about the anti-cancer potential of Dandelion Root Extract (DRE). This research proposal is based on our preliminary observation that DRE can induce cancer cells to commit suicide. It has been shown to reduce the number of cancerous cells in leukemia patients. This is a collaborative proposal involving a group of biochemists at the University of Windsor and an oncologist and clinical researcher at the Windsor Regional Cancer Centre. Our project aims are to: a) find out how this extract kills cancer cells; b) isolate the fraction of extract that has anti-cancer activity; and c) evaluate its efficacy in eliminating cancerous cells from samples obtained from leukemia patients including Chronic Myelomonocytic Leukemia (CMML)/Myelodysplastic Syndrome (MDS) patients and d) evaluate its efficacy in animal model of leukemia. The proposed research could lead to the development of a novel natural extract-based treatment of blood cancer.

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 leukemia by a non-toxic plant extract. Furthermore, identification and characterization of the active component of the DRE would lead to the possibility of concentrating, and thus making a better formulation, of the extract to be used for treatment. The biochemical knowledge of how this extract kills cancer cells could transform the understanding of natural compounds as chemotherapeutics and open a new window of opportunity to treat blood cancer with non toxic natural extract.

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PROGRESS REPORT

1A: Objective 1: *To evaluate the efficacy of different fractions of DRE in terms of selective induction of apoptosis in various commercially available human leukemia cell lines, including chronic myelomonocytic leukemia/ myelodysplastic syndrome (CMML/MDS) cells lines and in non-cancerous cells.*

We have completed this part of the work and I am happy to report that we have shown for the first time very high apoptosis inducing activity of DRE against three different CMML cell lines (MV-4-11, U-937 and HL-60 cells). We discovered that a repeated treatment with low dose was effective in killing most of the cancerous cells. More importantly, we have observed the selectivity of DRE, as it has shown no significant toxicity on non-cancerous cells (NHFs and ncPBMCs). We have published these findings in *PLoS ONE* journal.

In order to investigate whether the anticancer activity of DRE is limited to only haematological cancers or it is effective against other cancer cell lines, we performed similar experiments with different cancer cell lines. We have observed that DRE was effective in killing other aggressive cancer cells including Human Melanoma, colon and pancreatic cancer cell lines. We also studied the effect of other natural compounds such as Curcumin and piperlongumine.

1A: Objective 2: *To investigate the biochemical mechanism of apoptosis induction by DRE using in-vitro models.*

We are continuing to work on the investigating the mechanism of action of DRE for induction of apoptosis in cancer cells. The results obtained suggest that the mechanism of action is similar in the three different cancer cell lines that have been studied (see the attached research papers). Interestingly, we have observed that DRE was effective against two most aggressive human cancer cell lines (human melanoma and pancreatic cancer cells). Our research paper published in ECAM was featured in Washington Post (see the attached clip) in April last year. The MS with results of pancreatic cancer cells has been published in *PANCREAS*.

A major effort was made and is still on going in the determination of bioactive component(s) of DRE. We have fractionated an ethanolic extract of dandelion root to identify the fraction/compound(s) responsible for anti-cancer activity. In collaboration with Dr. Arnason at the University of Ottawa, we have prepared 193 fractions and each is being analyzed for activity and chemical composition. We have analyzed these fractions for anti-cancer activity and have found three pools of fraction with very high bioactivity. These selected fractions were further resolved by HPLC to obtain sub-fractions with higher activities. We are hoping to obtain LC-MS data for these fractions to get an idea of compounds present in them. ***This part of the work will continue in future years.***

1A: Objective 3: *To assess efficacy of DRE alone or in combination with Histone Deacetylase (HDAC) inhibitors in ex-vivo assays with patient-obtained leukemia samples (CML, AML, and CMML/MDS).*

We have obtained ethical approval from Windsor Regional Hospital and University of Windsor in order to obtain blood samples from newly diagnosed patients. We have completed this experiment with ten patients so far. Results are very encouraging. Almost all leukemia samples obtained responded very well to DRE, alone and in combination with valproic acid (a known HDACi). In parallel, obtained peripheral nucleated blood cells from healthy volunteers were used as non-cancerous ex-vivo models and we evaluated the toxicity (if any) of DRE. Our results indicated no toxicity to the non-cancerous cells. ***This work will continue in future years; to confirm***

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PROGRESS REPORT *cont'd*

this observation, we would need more samples for observation and publication of this work. Our preliminary data with 10 patients samples is encouraging.

1A: Objective 4: *To assess the efficacy of DRE in in-vivo mouse models with xenotransplants of human leukemia and other cancers.*

This part of the work was done in the second year. We have evaluated toxicity of DRE in mice (non-cancerous) by an oral regimen of DRE for a month. With our initial experimentation, we have observed that DRE was well tolerated in mice; no weight loss was observed and tissue samples were stained and analyzed. We did not see any evidence of toxicity in the control or the treated mice.

We have completed two sets of experiments with human colon cancer xenotransplants in immunocompromised mice to see the effect of DRE *in vivo*. All the results are described and shown in the Investigator's Brochure as a part of our application to Health Canada for Clinical Trial Approval.

1B: CLINICAL TRIAL APPLICATION (HEALTH CANADA)

In the past 6-8 months, we focused on preparing our application to Health Canada for the approval of a Phase-1 clinical trial. We needed to collect data and do experiments to obtain results needed for the CTA application. We needed to provide the heavy metal, pesticides and microbial content analysis of DRE, as well and *in vivo* data, to Health Canada reviewers. With Dr. Hamm as the lead clinical investigator and all the oncologists at the WRCC, we submitted the application in June 2012. **We are pleased to report that Health Canada has granted us approval for Phase-1 clinical trial of DRE for haematological malignancies at the WRCC.**

For more information, please visit the links below:

http://www.nlm.nih.gov/medlineplus/druginfo/natural/706.html
http://www.umm.edu/altmed/articles/dandelion-000236.htm
http://www.umm.edu/altmed/articles/dandelion-000236.htm#ixzz1sDRSFq5P

Conference Presentations:

- Ovadje P, Guerrero JA, Hamm C, Arnason JT, Pandey S. The Effectiveness of Dandelion Root Extract against Aggressive and Non-Responsive Cancers. **1st Annual WCRG Research Conference, Windsor ON, Canada**, November 17, 2012 (PhD work, Poster Presentation)
- Siyaram Pandey, Pamela Ovadje, Dennis Ma and Caroline Hamm. Selective induction of cancer cell death by natural products from dandelion root, turmeric and long pepper extracts. **Invited speaker International Conference on Cultivating Natural Bioactives for Health and Disease, University Of Western Ontario, London, ON**. July 9-11, 2012.
- Ovadje P, Hamm C, Pandey S. Evaluating The Efficacy Of Dandelion Root Extract as an Anticancer agent In Highly Aggressive and Resistant Cancers. **Natural Bioactives International Conference, London ON, Canada**, July 9 - 12, 2012 (PhD work, Oral Presentation).

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PROGRESS REPORT *cont'd*

- Ovadje P, Hamm C, Pandey S. Evaluating The Efficacy Of Dandelion Root Extract as an Anticancer agent In HighlyAggressive and Resistant Cancers. The **9th Annual NHP conference and Trade show, Kelowna BC, Canada**, May 22-25, 2012 (PhD work, Oral Presentation)
- Ovadje Pamela, Hamm Caroline and Pandey Siyaram. Evaluating the efficacy of Dandelion Root Extract in aggressive and non-responsive cancers. **Canadian Cancer Research Conference Toronto, ON, Canada**, Nov 24-27, 2011 (PhD work, Poster Presentation)
- Ovadje Pamela, Hamm Caroline, Pandey Siyaram. Evaluating the efficacy of Dandelion Root Extract in human Chronic Myelomonocytic Leukemia cells. **8th Annual Natural Health Products Research Society Conference "Multidisciplinary Approaches to Modern Therapeutics", Hilton Montreal Bonaventure, Montreal, QC, Canada**, May 24-27, 2011. Platform presentation.
- Madona Chochkeh, Pardis Akbari Asl, Pamela Ovadje, Siyaram Pandey (2011) Selective induction of autophagy and apoptosis through treatment with Dandelion Root Extract in human pancreatic cancer. **8th Annual Natural Health Products Research Society Conference "Multidisciplinary Approaches to Modern Therapeutics", Hilton Montreal Bonaventure, Montreal, QC, Canada**, May 24-27, 2011. Platform presentation

Manuscripts:

- Pamela Ovadje, Caroline Hamm and Siyaram Pandey (2011) Efficient induction of extrinsic cell death by Dandelion Root Extract in human Chronic Myelomonocytic Leukemia (CMML) cells. PLoS ONE 7(2): e30604. doi:10.1371/journal.pone.0030604.
- Pamela Ovadje, Madona Chochkeh, Pardis Akbari Asl, Caroline Hamm and Siyaram Pandey (2012) Selective induction of apoptosis and autophagy through treatment with Dandelion Root Extract in human pancreatic cancer cells. PANCREAS, May 27, 2012. doi: 10.1097/MPA.0b013e31824b22a2.
- Sudipa June Chatterjee, Pamela Ovadje, Caroline Hamm and Siyaram Pandey (2011) The Efficacy of Dandelion Root Extract in Inducing Apoptosis in Drug-resistant Human Melanoma Cells. In Press Evidence-based Complementary and Alternative Medicine (eCAM), doi:10.1155/2011/129045. Published online ahead of Print.
- Ovadje, P., Chatterjee S., Griffin C., Tran C., Hamm, C., and Pandey, S (2011). Selective Induction of Apoptosis through Activation of Caspase-8 in Human Leukemia cells (Jurkat) by Dandelion Root Extract. J. Ethnopharmacology, 133 (1): 86-91.

Research Collaborations and Grants:

Dr. Arnason, a well-known phytochemist at the University of Ottawa, is our active collaborator. We are working together on the chemical analysis of DRE. In addition, I have signed an MTA document to start collaborating with Dr. Ping Dou at the Karmanos Cancer Centre in Detroit MI.

Highly Qualified Personnel Training:

- Pamela Ovadje: A doctoral student has been working with 100% effort on this project. She will be graduating in next two years.
- Sudipa June Chatterjee: A M.Sc. student working with 25% effort on this project has graduated in 2010.
- Madona Chochkeh: A B. Sc. Hons thesis Student working on this project has graduated in 2011.