Adjuvant melatonin in the prevention of recurrence and mortality following lung cancer resection (AMPLCaRe): a randomized placebo controlled clinical trial

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1. Background and Rationale

Lung cancer accounts for the greatest incidence of cancer mortality worldwide; more than colon, gastric, prostate and breast cancer combined, in men and women respectively [1]. According to a 2002 report by the International Agency for Research on Cancer, lung cancer was responsible for 1.18 million deaths yearly [2]. Non-small-cell lung cancers (NSCLC) comprises the vast majority of cases accounting for 80% of lung cancer diagnoses [3]. Surgical resection followed by adjuvant platinum-based chemotherapy remains the standard of care, offering the best chance for long-term care in patients with resectable disease who can tolerate surgery [4, 5]. Despite optimal therapy, however, loco-regional or systemic recurrence occurs in an unacceptably high proportion of patients, leading to mortality in the vast majority of those patients [6].

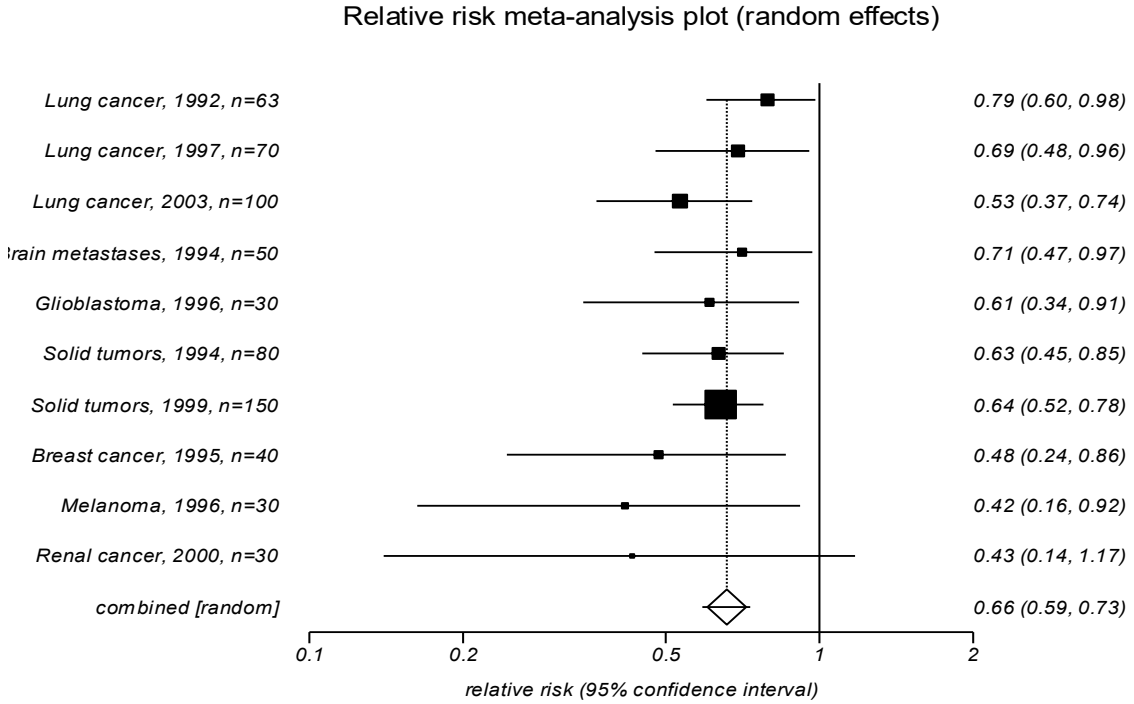
A recently validated prognostic index designed for NSCLC gives some measure of expected mortality in patients with this disease [3]. The patient's index is scored based on performance status, age, sex, histological type, and TNM staging. Using this index system and dividing the risk strata into five categories, the rates for 2-year mortality in each respective quintile are: 1st: 39%; 2nd: 72%; 3rd: 81%; 4th: 93%; and 5th: 96% [3]. Using the more standard International System for Staging Lung Cancer, 2-year survival based on initial diagnosis of clinical disease stage is: IA: 82%; IB: 59%; IIA: 57%; IIB: 43%; IIIA: 27%; IIIB: 15%; and IV: 10% [7].

Natural health products (NHPs) consist of a broad class of products that include vitamins, minerals, herbal remedies, homeopathic medicines, traditional medicines, probiotics, and other products like amino acids and essential fatty acids [8]. Despite limited scientific evidence for benefit or safety, a large proportion of patients with cancer do take NHPs, frequently without informing their physician [9, 10]. A large randomized controlled trial of antioxidant therapy with beta-carotene and vitamin E led to an increase in the risk of lung cancer [11]; as pharmacologic dosing of anti-oxidants potentially led to a paradoxical augmentation of other oxidants [12]. However, a more recent clinical trial found that the addition of vitamin C, vitamin E, and beta carotene with paclitaxel and carboplatin therapy had no effect on toxicity and showed a trend towards improved survival [13]. Two-year survival was 15.6% in the antioxidant combination arm vs. 11.1% in the chemotherapy arm. The study, was underpowered and conducted in a resource poor setting, however, and the results not found to be statistically significant ($p = 0.20$) [13]. Considering the use of adjuvant NHPs by the public, the interaction of NHPs with traditional cancer therapy, including surgery, chemotherapy and radiotherapy, are under increasing scientific scrutiny. Numerous investigators have called for the performance of large clinical trials to document the potential benefit or harm of NHPs used concurrently with conventional anticancer therapies [14-16].

Melatonin is a NHP that has demonstrated anticancer activity in the laboratory, in observational studies, and in randomized clinical trials [17, 18]. Specifically, melatonin is a promising candidate in the treatment and prevention of lung cancer. We previously conducted a systematic review and meta-analysis of all randomized controlled trials involving melatonin in the treatment of cancer. The meta-analysis combined relative risks of mortality at one year for nine separate clinical trials involving a number of solid tumour cancer trials (figure 1a). The pooled results were highly significant, with risk of mortality being reduced by 34% (RR: 0.66 (95% CI: 0.59, 0.73; $P \leq 0.0001$) [18], translating into a number needed to treat (NNT) of 4 (i.e. for every four

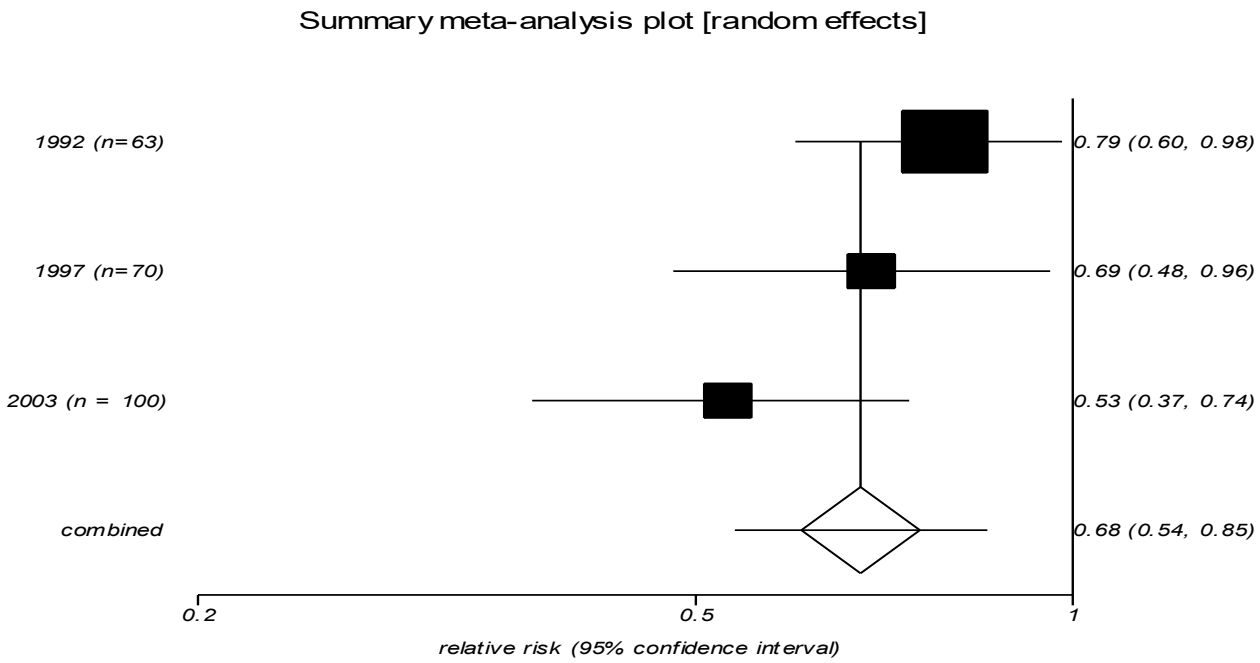
people treated with melatonin, one extra person would be alive at one year as a result of being given this intervention). These findings have been published by a single group of investigators based in Europe, and no independent study has been conducted in North America or elsewhere to confirm or discredit these encouraging findings.

Figure 1a: Meta-analysis of 1-year mortality in cancer patients given 10-50 mg of melatonin nightly



By restricting the meta-analysis to a subset of trials in non-small-cell lung cancer (NSCLC), a similar result was obtained. As shown in figure 1b, the relative risk of one-year mortality was reduced by 32% (RR: 0.68 (95% CI: 0.54, 0.85; P ≤ 0.0001) translating, again, to a NNT of 4 [19]. Although the same network of investigators conducted these studies, it is encouraging to note that the overall quality of the trials is fair, and no conflict of interest was evident. It is noteworthy that melatonin is not a patentable natural health product.

Figure 1b: Subgroup meta-analysis of 1-year mortality in NSCLC given 20 mg of melatonin nightly



Considering the use of adjuvant NHPs by the public, the interaction of NHPs with traditional cancer therapy, including surgery, chemotherapy and radiotherapy, are under increasing scientific scrutiny. Numerous investigators have called for the performance of large clinical trials to document the potential benefit or harm of NHPs used concurrently with conventional anticancer therapies [11-13]. There is some evidence to suggest potential synergistic effects when melatonin is combined with chemotherapy [14, 15]. The antitoxic effects of melatonin on chemotherapy has been demonstrated in some clinical studies [10, 16, 17] and in a recent update of our systematic review, where we found that the addition of MLT to chemotherapy significantly reduced incidence and/or severity of asthenia, leucopenia, nausea and vomiting, hypotension, and thrombocytopenia, while decreasing one-year mortality (RR 0.60, 95% CI, 0.54-0.67) [18]. For impact on both cancer mortality and side effects of chemotherapy, however, confirmation and validation is needed from independent and methodologically rigorous clinical research. We seek to investigate the safety and efficacy of melatonin through a well-designed and adequately powered trial. With the proposed phase II substudy, we will increase the power of the trial overall and explore the issue of physiological impact in critical areas of importance.

In a recent update of our systematic review and meta-analysis of all peer-reviewed controlled clinical trials we found a consistent effect on reductions of mortality and an additional impact on toxicities associated with chemotherapy [20]. The outcomes analyzed included the effects of MLT in conjunction with chemotherapy, radiotherapy, supportive care, and palliative care on: one-year survival, complete response, partial response, stable disease, and chemotherapy associated toxicities. We included data from 21 clinical trials. The pooled RR for one-year mortality was 0.63 (95% CI, 0.53-0.74, P<0.001). Improved effect was found for complete response, partial response, and stable disease with RR of 2.33 (95% CI, 1.29-4.20), 1.90 (1.43-2.51), and 1.51 (1.08-2.12), respectively. In trials combining MLT with chemotherapy, adjuvant MLT decreased one-year mortality (RR 0.60, 95% CI, 0.54-0.67) and improved outcomes of: complete response, partial response, and stable disease; pooled RRs were 2.53 (1.36-4.71), 1.70 (1.37-2.12), and 1.15 (1.00-1.33), respectively. The addition of MLT to chemotherapy also significantly reduced incidence and/or severity of asthenia, leucopenia, nausea and vomiting, hypotension, and thrombocytopenia.

1.2. Melatonin physiology and anticancer activity

Melatonin is endogenously produced primarily by the pineal gland from a dietary amino acid precursor, L-tryptophan, and then released into circulation. There are other organs and tissues that can synthesize melatonin and that also contain high relative levels of this molecule however the majority of endogenous production is due to the pineal gland [21]. In the pineal gland, melatonin synthesis is controlled by enzyme activity regulated through exposure to retinal UV radiation and a requirement for dark conditions to allow production and release to proceed [22].

Melatonin acts on cell membrane receptors and a nuclear site of the retinoic acid receptor superfamily. The two cell surface receptors, MT1 and MT2, have a high affinity for melatonin and belong to the G-protein-coupled receptor family. These receptors are found in virtually all cells including lymphocytes.

There is a large and growing body of evidence available from preclinical models that melatonin may reduce the incidence of cancer and indeed slow neoplastic cell growth [23, 24]. There are numerous mechanisms through which melatonin is believed to have an oncostatic effect and this is developed in much greater detail in a recent review by Mediavillia et al [25]. These anti-cancer actions include: a) antioxidant and anticarcinogenic effects; b) hormone receptor regulation; c) estrogenic modulation; d) modulation of cell cycle and induction of apoptosis; e) inhibition of telomerase activity; f) inhibition of metastasis; g) prevention of circadian disruption; h) antiangiogenesis; i) epigenetic effects; j) stimulation of cell differentiation; and k) activation of the immune system [25].

Melatonin Pharmacology and Pharmacokinetics

Maximal lifetime production of melatonin is achieved between the ages of one and three years old [26]. After three years of age, melatonin production begins to decline with peak nocturnal levels ranging between 50 and 250 pM [27, 28]. Normal melatonin production over the course of one day in healthy adults is approximately 28 μg with peak concentration levels in circulation averaging approximately 100 pM [29].

A wide range of doses from 0.1 to 40 mg or more are taken as over the counter medication and it is useful to consider the concentrations achieved after this level of exogenous administration. Pharmacokinetic studies employing oral administration of melatonin demonstrate that 0.1 to 0.3 mg given during the day led to peak serum levels consistent with nighttime peak levels [30]. Doses of 1 to 5 mg of melatonin resulted in plasma concentrations 10 to 100 times the usual nighttime peak [30]. In a high dose study 100 mg was given to healthy adults resulting in a serum concentration of 435 nM one hour following oral administration [31]. A serum concentration of 435 nM corresponds to a concentration approximately 4,000 times greater than that reached during normal peak nocturnal levels.

Melatonin is a small amphipathic molecule that easily traverses the blood brain barrier and is taken up by all compartments in the body. When taken orally, at doses between 2 and 4 mg, melatonin has an absolute bioavailability of approximately 15% [32]. Exogenous melatonin undergoes extensive and rapid metabolism with about 30-60 % of the melatonin being rapidly metabolized by the liver in a first pass effect [33-35]. Melatonin appears to be a selective substrate for CYP1A2 and possibly CYP2C19 [35-37]. It is first hydroxylated and then undergoes sulphate conjugation with primary excretion occurring in the urine [33, 35]. The elimination half-life of melatonin after exogenous administration is between 30 and 60 minutes [32, 38]. With respect to melatonin, males and females demonstrate similar pharmacological profiles [39], however, elderly women appear to have higher levels than elderly men [40]. Synthesis and release also varies with age and season; decreasing with advancing age and increasing during the winter months [41, 42].

Mechanism of Action

Many mechanisms have been proposed for the anticancer effect of melatonin, including antioxidant effects, estrogenic modulation, antimetastatic and antiangiogenic effects, prevention of circadian disruption, and particularly on the modulation of inflammation and immune function. However, very little work has been done to explore a mechanistic pathway and serological surrogates for melatonin's effects in NSCLC patients [25]. We seek to expand the parameters of the AMPLCaRe study in order to investigate the possibility that melatonin exerts its anticancer effects by regulating mediators of systemic inflammation and immune activation: NK cell function, cytokine levels, CRP, albumin, and leukocytes. These markers have been shown to be of good prognostic value in patients with NSCLC [43-49], but it is not known to what extent they may be modulated by melatonin.

Natural Killer (NK) cells

Natural killer (NK) cells are large, granular, bone marrow-derived lymphocytes that discriminate between normal healthy cells and abnormal cells by using a sophisticated repertoire of cell surface receptors that control their activation, proliferation and effector functions [50, 51]. These receptors are encoded by germline genes that do not require somatic recombination and thus categorize NK cells as specialized effector cells of the innate immune system. As integral members of the innate immune system, NK cells are involved in direct killing of cells displaying abnormalities linked to infection, malignancy or transplantation [51]. In addition to playing a role in antiviral responses, as highlighted by many groups, there is abundant evidence to suggest that NK cells play a crucial role in anti-cancer responses [50]. Depressed NK cell function has been observed in cancer patients [52, 53], and improved NK cell cytotoxic activity after surgery

and chemotherapy has been shown to correlate well with cancer survival [54, 55]. Importantly, melatonin has been shown to stimulate NK cell activity in vivo [56, 57], which may represent a key mechanism through which melatonin improves cancer progression and survival.

Cytokines

Cytokines are chemical mediators of inflammation, and may serve as surrogate of cancer related inflammatory activity in the body. IL-6, for instance, has been shown to be elevated in lung cancer patients, and this has been associated with decreased survival [44]. IL-2, on the other hand, is an immune stimulant and has been used as an immunotherapy for cancer patients [58, 59]. Cytokine interactions are highly complex, as can be seen from the below summary [60, 61]; hence it is important to assess them in combination as a complete panel:

- *IL2* - pro-inflammatory cytokine important for the proliferation of T, B and NK cells;
- *IL4*, *IL6, IL10* - regulate cell growth and differentiation and exert a suppressive effect on the immune response;
- *IL12* and *IL15* - pro-inflammatory cytokines important for the activation of NK cells;
- *IFN-γ* - secreted by T cells and NK cells with antiviral and anti-tumour properties;
- *GM-CSF* - pro-inflammatory cytokine that controls the production, differentiation, and function of granulocytes, macrophages and dendritic cells, in turn activating NK cells;
- *TNF-α* - pro-inflammatory cytokine secreted by macrophages; involved in the regulation of cell proliferation, differentiation, and apoptosis;
- *IFN-α* - pro-inflammatory cytokine secreted by all nucleated cells. Plasmacytoid dendritic cells secrete IFN-α in the early innate response to activate NK cell function;
- *TGF-β1* - controls proliferation, differentiation and contributes to suppressing NK cell function;
- *VEGF* - peptide active in angiogenesis, lymphangiogenesis, and endothelial cell growth. [60, 61]

C- Reactive Protein

C- reactive protein (CRP) is an acute phase protein and well accepted serologic marker of systemic inflammation. Recently CRP has been the subject of much research as a marker of lung cancer prognosis and survival. Cancer is a chronic inflammatory condition, and CRP may possess value as a marker of the tumor microenvironment. Elevated CRP has been associated with tumor size and lymphovascular invasion, and reproducibly predicts survival of NSCLC patients following surgical resection [43, 62, 63]. In one study, those patients with a pre-operative CRP level >10 mg/L had a median survival of 26.2 months, while those with CRP ≤ 10 mg/L survived for a median of 75.9 months ($p<0.05$) [43]. Similar findings have been reported for the prognostic value of post-resection measures of CRP. Meanwhile, Jones found that NSCLC patients with either elevated fibrinogen (>5 g/L) or CRP (>40 mg/L) (both acute phase proteins) had almost triple the risk of incomplete resection compared to those with values below this level, 23% compared to 8% ($p=0.09$) [47].

The ability of melatonin to impact CRP levels is not clear, however preliminary evidence suggests that lowered amplitude of nocturnal melatonin secretion is associated with higher IL-6 and CRP secretion [64, 65], suggesting a role for melatonin in the regulation of these cytokines. Importantly, reduced basal secretion of melatonin has been documented in patients post-

operatively [66]. *In vivo* studies show that high doses of melatonin can inhibit burn trauma-induced elevations in CRP [67], but similar findings in humans are lacking. A pilot study reported that melatonin was able to decrease levels of IL-6, the primary driver of CRP production, in a mixed cohort of patients with solid tumors or autoimmune disease [68], and a trial by Del Fabbro is in the process of assessing the impact of melatonin administered to gastrointestinal and lung cancer patients on CRP levels [69]. No other human trials to date have assessed this endpoint in this particular population.

Albumin

Albumin is the primary circulating osmotic protein and an accepted marker of protein deficiency and malnutrition. In cancer patients, the inflammatory state generated by the tumor exerts a profound catabolic effect on the body, and cancer patients often exhibit inversely related levels of CRP and albumin [49, 70]. According to a systematic review by Gupta, nine of ten observational studies demonstrated an inverse relationship between serum albumin and survival in cancer patients [46]. The Glasgow Prognostic Score is an inflammation based cancer prognostic index that is increasingly used in the area of cancer research; this index combines measurement of CRP and albumin levels as a way to quantify disease activity and predict survival [49]. If melatonin modulates systemic inflammation through IL-6 and CRP, it is conceivable that it may offset a cancer related decline in albumin levels and improve prognostic scores, however there is a present need for human level evidence to confirm such a mechanism.

Leukocytes

Like the inflammation-based GPS, leukocyte ratios are being increasingly utilized for their cancer prognostic value. Higher neutrophil: lymphocyte ratio (NLR) and platelet: lymphocyte ratio (PLR) have been correlated with inflammatory markers and hypoalbuminemia [49, 71], and have been shown to possess independent prognostic ability in lung and pancreatic cancer patients respectively [49, 72, 73]. In our updated systematic review, we found that melatonin reduced chemotherapy related leukopenia and thrombocytopenia [68], suggesting that this may be a mechanism for melatonin's effects on survival, however this relationship has not been directly studied in humans. As a Complete Blood Count (CBC) test is needed for the leukocyte ratio, CBC will also be collected for this study.

Preliminary Work

In addition to the systematic reviews and meta analyses of adjuvant melatonin therapy cited above [18, 20], our team is involved in specialized work in the field of NK cell function, especially as it pertains to tumor biology. NK cells are promising as targets for cancer immunotherapy, since NK cells act as an intrinsic surveillance system involved in the direct killing of cells displaying malignant abnormalities [51]. Our work has demonstrated that NK cells are essential for the removal of tumor metastases, and that the immune stimulant Poly I:C can boost NK cell function, resulting in better attenuation of experimental lung metastases in murine models (**Figure 1**). To directly assess NK cell function, we have used novel cytotoxicity assays to show that Poly I:C was able to increase NK cell cytotoxicity against YAC-1 tumor targets *in vitro* (**Figure 2**), and we have furthermore demonstrated NK cell cytotoxicity against K562 tumor targets from human blood (**Figure 3**). In addition, we can characterize heightened NK cell function by identifying cell surface markers through flow cytometry.

Melatonin is well established as an immune stimulating agent [74], and has been shown to stimulate NK cells *in vivo* [56, 57]. Through this project we aim to combine expertise in these distinct areas in order to conduct a new and important study into the impact of melatonin on NK cell function in cancer patients, as well as on immune function in general, which will be measured by the other surrogate markers planned.

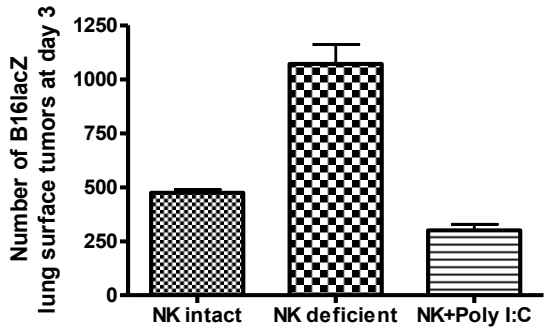


Figure 1. Natural killer cells are essential for the attenuation of lung tumor metastases following surgery. All C57Bl/6 mice were challenged intravenously with 1e6 B16lacZ melanoma tumor cells. The NK deficient group received anti-asialo antibody given i.v. to deplete NK cells prior to B16lacZ tumor inoculation. The NK + Poly I:C group received 150ug of Poly I:C intraperitonealy to boost NK cell activation prior to B16lacZ tumor inoculation. Animals were euthanized at day 3 following tumor cell injection. Lungs were harvested and stained with X-gal to visualize pulmonary metastases.

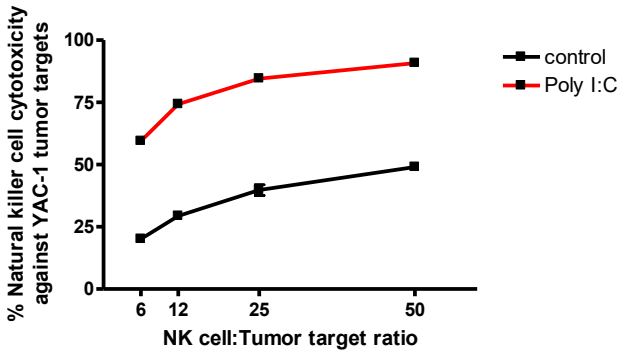


Figure 2. Poly I:C enhances NK cell cytotoxicity against tumor targets - 18h post treatment, purified NK cells were isolated and pooled from the spleens of either control (PBS) or Poly I:C (150ug i.p.) injected C57Bl/6 mice. These were used as effector cells in cytotoxicity assays againt chromium-51 labelled YAC-1 tumor targets as the indicated NK:Tumor target ratios. Chromium release was measured with a gamma counter after 4h.

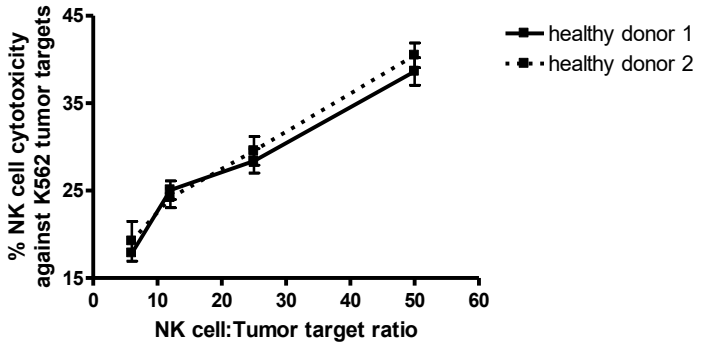


Figure 3. Human NK cell cytotoxicity can be reproducibly measured against tumor targets. PBMCs were isolated from whole blood of two healthy human donors. These were used as effector cells in cytotoxicity assays against chromium-51 labelled K-562 tumor targets as the indicated NK:Tumor target ratios. Chromium release was measured with a gamma counter after 4h.

2. Study Objectives

2.1 Primary Objectives

1. To determine the effectiveness of adjuvant melatonin as compared to placebo in the prevention of cancer recurrence and mortality two years after surgical resection of NSCLC.
2. To explore the effect of melatonin on promising biological markers within participants enrolled in the larger clinical trial. These markers include the most promising assays that address areas in which melatonin may act against cancer. The assays include NK cell function and cytokine levels. In addition to addressing the very important issue of mechanism of action, the additional 80 participants will further contribute to the power of the phase III trial.

2.2 Secondary Objectives

1. To assess the impact of melatonin compared to placebo on the frequency, severity, and type of adverse effects caused by post-operative adjuvant chemotherapy and/or radiation.
2. To assess the impact of adjuvant melatonin compared to placebo on quality of life (QOL), sleep, pain, anxiety, depression, and fatigue.
3. To determine the type and presence of any adverse effects induced by melatonin in comparison to placebo by chart review and interactions with patients at their study visits.
4. To compare the time to recurrence and time to mortality (up to five years) between the placebo and melatonin groups.

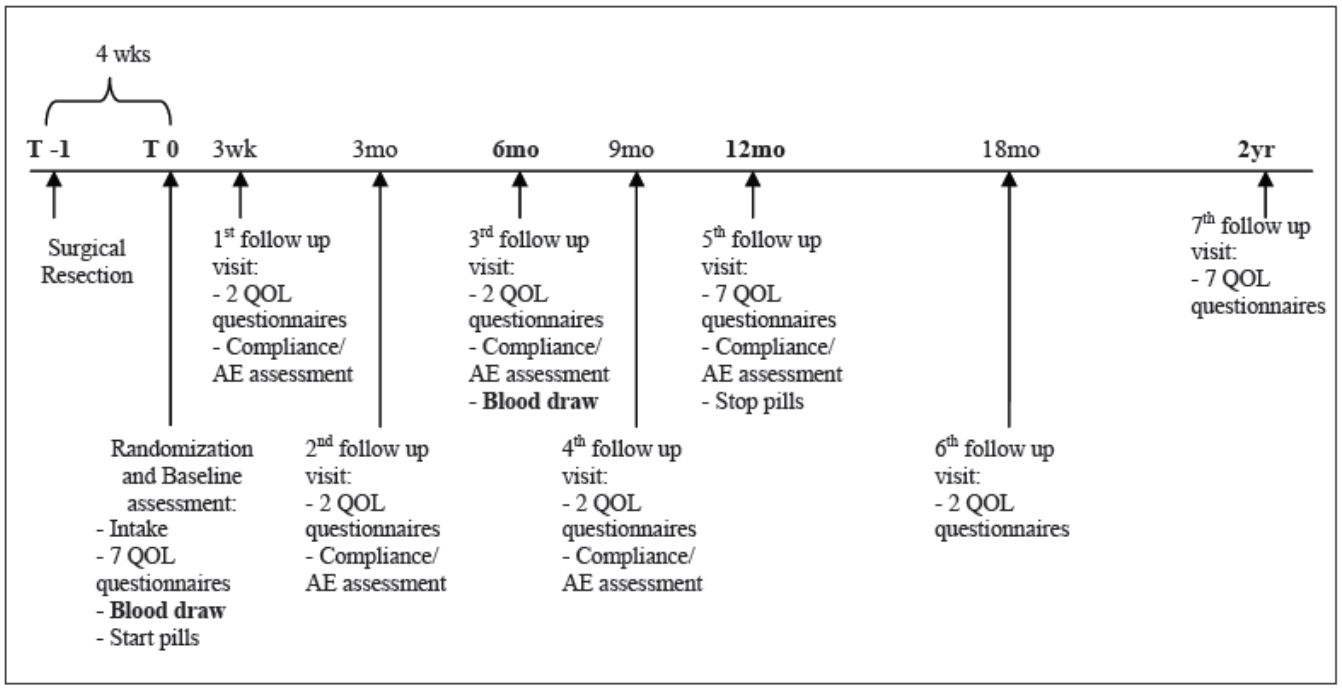
3. Hypotheses

a) Adjuvant melatonin will reduce the incidence of lung cancer recurrence of NSCLC two years after surgical resection.
b) Adjuvant melatonin will reduce disease specific 2-year mortality following lung cancer resection.
c) Melatonin will lead to a reduction in chemotherapy related toxicities and have no impact on adverse effects due to radiation therapy.
d) Melatonin will lead to improved QOL, less fatigue, improved sleep, and have no impact on depression or anxiety in patients following lung cancer resection.

e) Melatonin will increase NK cell activity of people who are ingesting this at a dose of 20mg daily.
f) Melatonin will create a decrease in both inflammatory and anti-inflammatory cytokines including: IL4, IL6, IL10, IL12, IL15, IFN-a, GM-CSF, TNF-a, IFN-a, TGF-b1, and VEGF in people who are ingesting melatonin at a dose of 20 mg daily.

4. Study Design

The study is a two-arm, randomized, placebo-controlled, blinded trial among 640 subjects who have been diagnosed with NSCLC and are scheduled for surgical resection. Of these 640 patients 80 patients at the Ottawa Hospital will be included in the biomarker sub-study. Patients will be recruited into the main trial at the Ottawa Hospital as well as other centers across Canada.



NOTE: Past the two year follow up (until a maximum of five years), patients will be followed for recurrence date and/or date of death (using medical records, patient follow-up, and family follow-up). No questionnaires will be given past the 2-year follow up.

5. Eligibility Criteria

5. 1 Inclusion Criteria

1. Adult males or females with a clinical diagnosis confirmed by pathology of Stage I, II, III, or IV primary NSCLC who are eligible and scheduled for surgical resection.
2. Willingness to adhere to randomized treatment.
3. Availability for follow-up schedule of visits.
4. Ability to answer self- and interviewer- administered questions in English or French, or have an appropriate person available to assist with translation.

5. Understand and sign a written informed consent form in English or French, or have an appropriate person available to assist with translation.
6. For the sub-study only, willingness to have additional blood taken for study purposes.
7. Verbal confirmation from females of childbearing age that they are not pregnant, or documented birth control method or menopause. Breastfeeding patients will not be included in this trial.

5.2 Exclusion criteria

1. Taking exogenous melatonin for reasons related or unrelated to the diagnosis of lung cancer.
2. Synchronous lesions, or any situation where all cancer cannot be resected.
3. Pregnant and breastfeeding patients will not be included in the trial. A verbal confirmation that patients are not pregnant or breastfeeding has been deemed sufficient as the chance that patients from this population are either pregnant or breastfeeding is exceptionally low.

6. Site, Protocol, Methods and Procedures

6.1 Primary Site

The Ottawa Hospital is the Institution lead in this clinical trial. At this location, a multidisciplinary team will be responsible for the coordination of all the activities related to this study. The Ottawa Hospital is the only site involved in recruiting the additional 80 patients for the sub-study.

6.2 Participant Institutions

Centres wishing to participate in this trial are required to obtain full board local ethics approval of the protocol and consent form by the appropriate Research Ethics Board prior to study commencement. Centers are also required to submit any subsequent amendments to their Research Ethics Board and provide the coordinating center with approval letters. Any centre joining this trial must sign a Clinical Trial Agreement before trial commencement at their site. As per Good Clinical Practice guidelines, an annual renewal of the study is required by the participating center's research ethics board (unless otherwise specified).

6. 3 Recruitment and screening

Patients will be identified and selected prospectively from investigators' patient records. Entry into the trial may occur pre- or post-operatively, up to the point at which the trial intervention begins on the day of discharge from hospital up to 60 days after surgery. Even though there is a recruitment window of 60 days, it is important to enroll patients as soon as possible after surgery. Recruitment and patient accrual will occur during the first five years of the trial. To facilitate the enrollment REB approved informative brochures about the study may be placed in appropriate locations (i.e., exam rooms and waiting rooms). Recruitment for the 80 patients in the sub-study will be done post-operatively, as they will need to have a baseline blood draw at their first post-operative appointment.

The trial began enrollment in Oct 2007, and we have currently have eight sites including the Ottawa Hospital actively recruiting patients. Dissemination of the trial results will take place through publication in a high impact journal, through conference presentations, and to lung cancer patients via outreach to patient advocate groups and societies.

In all presentations of the phase III trial, the funding contributions of the Lotte and John Hecht Memorial Foundation and the Gateway for Cancer Research will be acknowledged. In all presentations of the phase II substudy, Gateway's sole contribution and support will be acknowledged.

6.4 Interventions

Patients who appear to meet eligibility criteria will be approached and asked to consent to the trial by the research coordinator. After eligibility criteria have been confirmed by the investigator or medically qualified delegate patients will be randomized using a centralized system at The Ottawa Hospital to either active melatonin group or placebo. Randomization will be stratified according to cancer staging (I/II vs. III/IV). Allocation bias will be prevented by using a randomization table with variable blocks of 4 and 6. Only the Pharmacy Research Technician at the Ottawa Hospital can see the randomization table and treatment assignments.

Patients, physicians, and the research coordinator will be kept strictly blinded as to individual group allocation and treatment. The intervention, active or placebo, will be given to participants in opaque bottles with the patient study code clearly written on the corresponding container. The clinical trial pharmacist at the coordinating site will be responsible for the coding and container labeling of all containers. Instructions for taking the intervention will be identical for both control and melatonin groups. The research coordinator will be responsible for the dispensing of the pills (both active and placebo).

Melatonin or placebo will be provided to patients either on the day of discharge or up to 60 days post-operative from the hospital. In the phase II substudy, participants will only be given the intervention four weeks following surgery. This wait period is to reduce the confounding effect of the surgery on the surrogate measures we are testing. Patients will be responsible for taking their doses and will be required to report any missed doses to the research coordinator. To ensure optimal compliance, the research coordinator will telephone each patient once a week for two weeks at the beginning of the trial. Telephone calls will then be made as needed until the completion of one year while the patient is on the study drug.

The intervention for those randomized to the melatonin group will involve the ingestion of 20 mg of melatonin taken as a single capsule nightly approximately one hour prior to bedtime. Melatonin has a soporific effect and thus nighttime administration is recommended [75]. The choice of 20 mg nightly is based on efficacy demonstrated using this dose in prior clinical research [18]. The Ontario based company, Promising Health Inc., will supply melatonin in the form of 20 mg capsules. Regulatory approval for the trial was given by the Natural Health Products Directorate (NHPD). In addition to confirming ethics approval, Health Canada's regulatory process requires strict approval regarding the chemistry and manufacturing of any NHP used in a clinical trial. Consumption of the melatonin will continue nightly for the first year of the trial.

The intervention for those randomized to the control group will receive an identical looking capsule in identical looking containers. Promising Health Inc. will also be providing the

placebo. Similar requirements for quality control and quality assurance will be required for the placebo as required for the melatonin.

6.5 Outcome Measures

The primary outcome is a composite outcome including either cancer recurrence and/or mortality within two years following the commencement of the intervention. Recurrence will be determined by clinical examination by the patient's thoracic surgeon in addition to radiological evidence of loco-regional or systemic recurrence of lung cancer. While contrast enhanced chest CT scan at one and two years post resection is the preferred radiological assessment, the standard of care radiological assessments for each site is acceptable. For study purposes, only chest CT is requested at 1 and 2 years, but results of head and abdominal CTs will be recorded if applicable. As per standard of care, lateral and P/A chest radiographs will be assessed at 3 weeks and at 3, 6, 9, 12, 18 and 24 months following hospital discharge.

Surrogate tests for the phase II substudy

A subset of 80 patients in both groups will undergo testing for NK cell function and characteristics, blood levels of cytokines, CRP, albumin, and leukocytes. Testing will occur four weeks following surgical resection but prior to commencing the study treatment (baseline) and six months after beginning the study treatment (end point). In addition to venipuncture for measurement of CRP, albumin, and leukocytes, immunological functional assays adapted by Dr. Auer's lab will be conducted on NK cell activity as well as performing a series of assessments for pro- and anti- inflammatory cytokines.

i. Human NK cell cytotoxicity assay

Human whole blood will be processed for Peripheral Blood Mononuclear cells (PBMCs) using Ficoll Paque (Stemcell). These will be used as Effector cells in an *ex vivo* NK cell cytotoxicity assay. The human NK cell sensitive tumor cell line – K562 (human leukemia) will be used as Target cells. K562 tumor cells will be harvested and labelled with 100μCi of ^{51}Cr (Perkin-Elmer) in the form of Na_2CrO_4 for 1 hour. They will be added to the PMBCs in the 96-well plate at different effector to target cell ratios (25:1, 12:1, 6:1, 3:1). The mixture will be incubated for 4 hours prior to analysis of chromium release in the supernatant using a gamma counter (Perkin Elmer). To show NK cell specific lysis of tumor targets, NK cells will be depleted from PBMC population using CD56 microbeads (Miltenyi Biotech) in a control experiment where no killing of targets will be detected.

ii. Cytokine array

Includes IL2, IL4, IL6, IL10, IL12, IL15, IFN-α, IFN-γ, TNF-α, GM-CSF, TGF-β1, and VEGF in a mix 'n match multi-analyte ELISA array (SAbiosciences, Qiagen). Serum (or plasma) will be obtained from human whole blood through centrifugation (30,000 rpm for 20mins). Human cytokines will be determined by sandwich enzyme-linked immunosorbent assay (ELISA), using the Mix 'n Match multi-analyte ELISA array from SAbiosciences. Briefly, 96-well microtiter plates arrive already coated with capture antibody. Recombinant antigens and patient samples are added to their respective wells and incubated for 2h. Samples are then washed and incubated with detection antibodies for 1h. They are again washed and incubated with Avidin-HRP for 30mins. Following a final wash, they are incubated with development substrate and stop solution and have optical density read at 450nm.

iii. Flow cytometry

PBMCs will be isolated from whole blood using Ficoll Paque (Stemcell). Blood lymphocytes will be analyzed for total number of NK cells using CD56 and CD3 fluorescent conjugated mAb. CD69 expression (early activation marker), IFNγ secretion (NK cell secreted cytokine in response to activation), CD107a (cell surface degranulation marker), and Granzyme B secretion (NK cell secreted cytoxtoxic granule) will be analyzed using specific fluorescent conjugated mAb. Events will be acquired on a Beckman Coulter CyAn and data analyzed using Kaluza software (Beckman Coulter, v1.1).

Secondary outcomes of the study include compliance, adverse events, time to recurrence and/or mortality, and scores from validated questionnaires to measure quality of life, pain, depression, anxiety, sleep, and fatigue. The following validated questionnaires will be used:

Questionnaire	**Acronym**	**Measure of**
Brief Pain Inventory	BPI	Postoperative pain
Beck Depression Index	BDI	Depression
Beck Anxiety Inventory	BAI	Anxiety
Leeds Sleep Evaluation Questionnaire	LSEQ	Sleep quality
Fatigue Symptom Inventory	FSI	Fatigue
Quality of Life in Cancer Patients	EORTC QLQ-C30	Quality of life
Quality of life in Lung Cancer Patients	EORTC QLQ-LC13	Quality of life specific in lung cancer patients

Note: the entire battery of questionnaires listed above will be administered at the Ottawa site at Baseline, 1 and 2 years. Only the Pain and Sleep questionnaires will be administered at 3 week, 3, 6, 9, and 18 months. At multicentre sites, the Sleep, Fatigue, QLQ-LC13 and QLQ-C30 questionnaires will be administered at Baseline, 1 and 2 years, and only the Sleep questionnaire at 3 week, 3, 6, 9, and 18 months.

Every document provided to the patients will be available in both English and French depending on language of preference. This also applies to the questionnaires, and licenses for each of them in both official languages have been obtained. Each of the questionnaires has been validated in both English and French.

Adverse effects attributed to chemotherapy and radiation therapy will be collected using the Common Terminology Criteria for Adverse Events (CTCAE version 3.0), a validated form published by the National Cancer Institute. During follow up visits Research Coordinators should look through the study diary with the patient and inquire about the severity of any adverse or serious adverse effects. During follow up phone calls, the severity of any adverse and serious adverse events should also be assessed.

6.6 Follow-up Calls/Visits and Data Collection

Information will be captured by the research coordinator who will fill in the case report forms and supervise patients' completion of the questionnaires at baseline, 3 weeks, 3 months, 6

months, 9 months, 1 year, 18 months and 2 years. Information regarding the results from chest x-rays or CT scans, chemotherapy and/or radiotherapy treatment, recurrence and past medical history/concomitant medications will be captured from the patient's chart. All other information will be captured by the research coordinator through telephone conversations and follow up visits with the patients.

The research coordinator will make a follow up phone call once per week for the first two weeks and then as needed for the one year while the patient is on the study drug. During telephone conversations, the research coordinator will assess study compliance and any adverse or serious adverse events. Information regarding these phone calls will be captured in the patient's study records.

At follow up appointments, the research coordinator will provide the patient with study drug refills (up to the 1-year mark), provide quality of life questionnaires, monitor any adverse or serious adverse events and monitor compliance. Compliance will be monitored during a brief interview, and by checking for left over pills. Patient self-report is an acceptable measure of compliance if counting pills is not feasible. Patients will be asked at each follow-up phone call and visit if they have missed any capsules. Patient reports of start and end dates of the capsules will also be used to determine compliance.

The study will be extended to include follow up periods past the initial follow up period of two years. Specifically, patient follow up times will be extended to include follow ups with all patients enrolled on study beginning in 2007. Patients' records will be tracked to determine whether they experienced a recurrence or death. Participants will be followed until they either reach the five year maximum or until 2 years after the last participant was enrolled in the study. As such, each patient will have a varying follow up period (with a range of 2-5 years).

Extension of follow ups past the two year time point will be conducted as follows:

Patients' medical records will be reviewed in order to record cancer recurrence or death. Should the information not be available in the medical records, their records will be requested from the Canadian Cancer Registry. If patient information cannot be found in the registry, surgeons will be asked to follow up with the patient's GP in order to find out whether they had a recurrence or died. If the patient's GP does not have this information, but directs us elsewhere, or if the medical records indicate that the patient was discharged to another facility (eg: another hospital), this facility will be contacted. At the facility's request, the signed patient consent form will be provided in order for them to release the information on the patient's death or recurrence. If this information cannot be retrieved through searching patient records in any of the above methods, patients will be contacted directly. Patient contact will begin with a letter and brief questionnaire to the last known address (letter and questionnaire provided by the coordinating centre) requesting an update on patient health status. If the patient does not respond to the letter, the study coordinator will contact the patient at their last known telephone number, and ask for a health status update. If the patient cannot be contacted, and all other options are exhausted, the study coordinator will contact the patient's next of kin (telephone script provided by the coordinating centre). Should we be unable to follow up in any of the above methods, participants

will then be considered lost to follow up. Sites should ensure that the letter, questionnaire, and telephone script receive REB approval prior to use.

Patients who 1) withdrew from the trial before starting the intervention, or 2) were enrolled but considered to be screen fails (did not meet inclusion criteria) and did not start the intervention, or 3) recurred within the first two years, will be followed only for outcome data (recurrence and/or mortality). The "Follow-up for Withdrawals and Screen Fails (who did not start intervention)" CRF will be completed annually for these patients (up to 5 years post-op). Adverse events will not be tracked for these patients.

6.7 Standardization

All study personnel, including surgeons, oncologists, nurses, and the research coordinator will undergo group familiarization and orientation of the study protocol to ensure comprehensive awareness of the study procedures. There will be an initial orientation for the study team at each site before the trial begins. Once recruitment is active and the trial is underway retraining and/or site visits will be performed as needed.

6.8 Data Management, Data Safety Monitoring Board (DSMB), and Trial Monitoring

Data Management: All data will be collected on case report forms by the research coordinator. The Methods Centre at the Ottawa Hospital Research Institute will be used to provide database design and data analysis services. The Coordinating Centre will be responsible for all aspects of the trial including day-to-day management.

DSMB: This study will be monitored by an independent DSMB, consisting of an independent epidemiologist, and three physicians not involved in this study. The DSMB will be immediately informed of any serious adverse events (SAEs), which are potentially related to study drug.

The DSMB is responsible for assuring that study participants in AMPLCaRe are not exposed to any unnecessary or unreasonable risks and that the study is being conducted according to the highest scientific and ethical standards. In order to carry out this responsibility, the DSMB will:

- Familiarize themselves with the Terms of Reference, study protocols, manuals and any amendments that are developed as the study progresses.
- Review descriptive reports and interim analyses.
- Provide recommendations for or comments regarding change(s) to the design or methodology of the clinical trial.
- Provide feedback on ancillary studies.
- Alert investigators regarding emerging procedural or ethical issues.
- Comment on the relevancy of new external published data from other trials that may impact on patient safety or efficacy of the study treatments.

DSMB members will review data only by masked study group (such as A vs. B rather than experimental vs. control). The DSMB may request unblinded safety data tables, in cases of adverse events associated with study drug or procedures. Serious adverse events, which are of

concern to members of the Steering Committee (potentially study drug related) will also be reviewed by the DSMB.

If at any point the DSMB considers continuance of the study unacceptable, the sponsor will be immediately notified.

Trial Monitoring: Monitoring will be conducted by qualified research personnel according to the trial monitoring plan developed in conjunction with OHRI administration. Essential regulatory documents as laid out in section 8 of ICH/GCP will be reviewed in addition to performing risk based source document verification.

6.9 Adverse Events

An "adverse event" means any adverse occurrence in the health of a clinical trial subject who is administered a natural health product, that may or may not be caused by the administration of the natural health product, and includes an adverse reaction, a serious adverse reaction and a serious unexpected adverse reaction.

According to the Health Canada approved product monograph, expected adverse events associated with melatonin include fatigue, nausea, vomiting, and cramping. Though rare, allergic reactions have been reported. Morning fatigue may occur with greater frequency at the beginning of the study [76]. Patients should be informed that melatonin should not be consumed with alcohol, other medications or natural health product with sedative properties.

In a review of scientific literature, in very rare cases (only 9 out of 307 articles reported adverse effects), the following reactions of Melatonin were reported:

- Four cases of seizures
- Two cases of skin eruptions (skin rash)
- One patient with autoimmune hepatitis (chronic disease of the liver)
- One case of confusion
- One case of optic neuropathy (disease of the eye)
- Four cases of fragmented sleep
- One psychotic episode
- One case of nystagmus (rapid involuntary rhythmic eye movement)
- One case of headache

Given the anecdotal nature of much of this evidence, however, it is debatable whether or not Melatonin was the true cause of the adverse events reported. The adverse effects of melatonin appear to rest on a limited number of studies and case reports. However, few studies have been conducted to establish the veracity of attributing these effects to melatonin.

Interaction of melatonin with nifedipine is perhaps the best documented interaction in that it has evidence from a randomized controlled trial, albeit a small one [77].

Patients taking nifedipine and concomitant melatonin showed a decrease in blood pressure control from baseline administration of nifedipine only. Therefore baseline and follow up

monitoring of blood pressure for patients taking nifedipine is warranted. This possible interaction will be flagged for all patients taking nifedipine with recommendations in the chart made to monitor for any unanticipated rise in blood pressure.

The incidence of all adverse events, expected and unexpected, and any steps taken to treat the complication will be documented in the patient's chart and study file and recorded on the Adverse Events case report form.. Adverse events should be communicated to the REB according to the REBs policies and procedures. The frequency and nature of serious adverse events will be documented and reported to the DSMB.

Adverse events will be tracked in the following situations:

- All patients on study up to the 2-year time point.
- Patients who started study capsules and withdrew within 2 years (will continue to follow radiographically, so AEs will also be tracked).
- Screen fails who started study capsules.

Adverse events will not be tracked in the following situations:

- Any patient beyond the 2-year follow up.
- Patients who recur within 2 years (will continue to be tracked for mortality only).
- Patients who did not start the study drug (screen fails or withdrawals).
- Prior to signing the consent form and starting the study intervention

Patients in this study may experience adverse events that would be considered expected for lung cancer patients. These events include the following:

- Pain
- Insomnia
- Fragmented sleep
- Lack of appetite
- Lung infection
- Pneumonia
- Shortness of breath
- Fatigue
- Depression/depressive symptoms
- Dizziness
- Cough

Furthermore, due to surgical complications, patients in this study may experience expected serious adverse events (considered serious by definition of prolonged hospitalization). These events include the following:

- Atelectasis
- Pneumonia
- Cardiogenic edema
- ARDS
- Empyema
- Effusion

- Hemothorax
- Prolonged air leak
- BP Fistula
- SubQ emphysema
- Atrial arrhythmia
- Ischemia
- Hypertension
- CHF
- Cardiac effusion
- UTI
- Renal insufficiency
- Non-infect. diarrhea
- Constipation
- Vomiting
- Ileus (bowel obstruction)
- Confusion
- Infection
- C-difficile
- Heart block

Patients in this study may also be undergoing chemotherapy or radiation treatment. As such it is reasonable to expect that they may experience adverse events related to this treatment, which include the following:

- Febrile neutropenia
- Nausea/vomiting
- Fatigue

It is possible that in some cases the events defined above may be related to the study drug (or exacerbated by the drug). The site investigator will assess the relationship of the study drug to the events listed above and the seriousness of the event (see 6.10 Serious Adverse Events)

Patients in this study could be diagnosed with a new primary cancer while on study. If this is the case, the new primary is reported as an adverse event. However, a recurrence of the lung cancer (outcome) will not be reported as an adverse event.

6.10 Serious Adverse Events (SAEs)

According to the principles of ICH GCP, a serious adverse event is any untoward medical occurrence that at any dose: results in death, is life threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/ incapacity, or is a congenital anomaly/birth defect. When a serious adverse event is identified, the Serious Adverse Event form should be completed and signed by the lead principal investigator at the site. Please refer to the AMPLCaRe Procedures Manual, or contact the coordinating center should you have any questions or concerns.

The Natural Health Product regulations state that all **adverse events deemed to be serious and related/possibly related to the intervention are subject to expedited reporting to Health Canada**. These events are referred to as **Serious Adverse Drug Reactions (SADRs) and can be either expected (SADRs) or unexpected (SUADRs) in nature**. Therefore, if an event is determined to be **serious, and related/possibly related to the intervention, regardless of expectedness,** it must reported to the coordinating center within 24hrs of becoming aware of the event and subsequently reported to the site REB according to their policies and procedures.

The coordinating center will notify Health Canada as soon as possible but no later than 7 calendar days after becoming aware of the event for fatal or life threatening events and no later than 15 days after becoming aware for non fatal/life threatening events. Please refer to the AMPLCaRe Procedures Manual, or contact the coordinating center should you have any questions or concerns.

6.11 Contraindications/Drug Interactions:

Patients who are pregnant or breastfeeding are excluded from participation in this trial. Inclusion of participants with a significant health conditions such as asthma, cardiovascular disease, chronic kidney disease, depression, diabetes or hypoglycemia, hormonal disorder, immune system disease, liver disease, migraines, seizure disorders will be considered on a case by case basis by the site investigator and coordinating center. In order to monitor for possible drug interactions, patients who are taking anticoagulants, anticonvulsants, blood pressure medications, immunosuppressive medications, sedative, hypnotic or psychotropic medications, or steroids will be advised by the research staff to advise their treating physician if they experience any change in their pre-existing conditions.

6.12 Premature withdrawal

Patients who withdraw prematurely from the study will be encouraged to return for a final visit. By adhering to an intention-to-treat model, patients who withdraw from taking the study drug prematurely will be asked if they would be willing to be followed radiographically (including possible recurrence or mortality) up to a maximum of five years past their date of enrollment . If the patient agrees, the Research Coordinator will document this in the patient's chart and will continue to record the results of the standard of care chest x-rays and CTs, and whether the patient had a recurrence or death. The patients will no longer be given questionnaires to complete if they withdraw.

Considering the standard procedures patients will be following for their regular care and that this trial does not require any additional frequency of visits, we do not anticipate this to be a major concern.

6.13 Privacy & Confidentiality

Patient's personal health information will be kept confidential, unless release is required by law. The consent form should make note that representatives of the local REB and/or institution, the Canadian College of Naturopathic Medicine (CCNM) and/or their designee, as well as Health Canada may review original medical records.

Patients enrolled in the study will not be identified in any publications or presentations resulting from this study, unless permission is given by the patient. All case report forms and associated source documents will be kept in locked cabinets and all databases will be password protected – both will be kept for a period of 25 years. Case report forms will be shredded and databases will be destroyed at the end of this retention period.

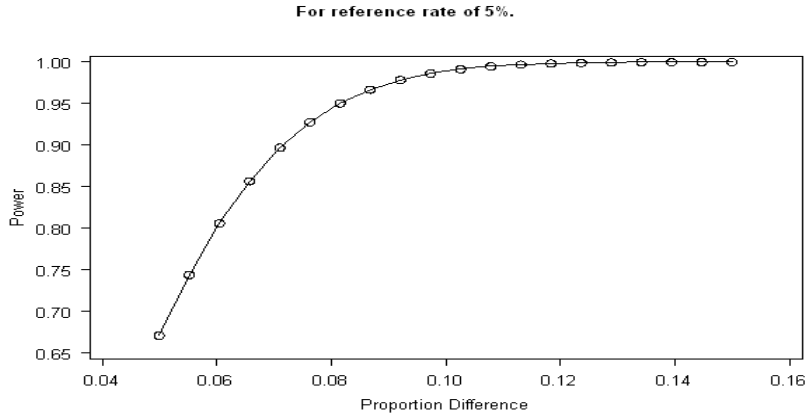
A password-protected file will be maintained at each participating center and will contain patients' names and corresponding unique study identifiers, only of individuals they have enrolled at their site. A second separate password-protected database will be maintained at the Coordinating Center and will contain information gathered from the case report form with the unique study identifier from all participating centers. This information will not leave the Ottawa Hospital.

7.0. Sample Size Determination

The tables and graphs below demonstrate the means through which we can achieve much greater power to detect a real difference by increasing the sample size to a total of 640.

Assuming that one arm of the trial (intervention or placebo) sees a combined event rate of 5% for the primary outcome of recurrence and mortality and our total N = 640 (320 per arm) then we will be able to see a proportional difference as low 6 to 7% with a power of 80 to 90% respectively (Figure 1). The initial proposal was to see an absolute difference of 15% in recurrence and mortality given 80% power. The addition of 280 participants (80 from the substudy and an additional 200 from this request) will enable a much greater resolution for a smaller benefit which is also clinically relevant. Most importantly if there is a real benefit to be seen then we will be much less likely to miss it with an expanded trial population.

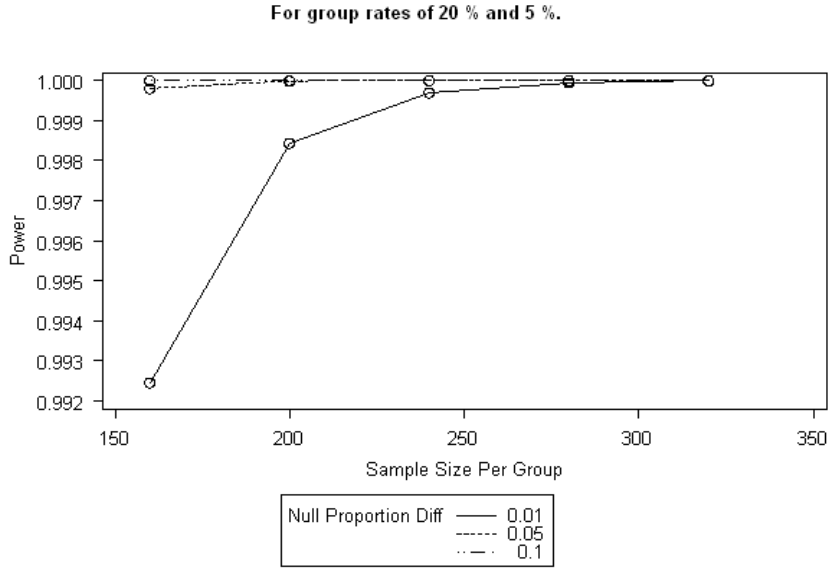
Figure 1: Graph of power versus rate difference to detect for a reference rate of 5 %. [So: with a reference of p1 = 5 %, a difference p2 - p1 = 0.07 or 7 % implies that p2 = 12 % and approximately 85% power] - 320 in each arm.



The next graph (figure 2), demonstrates that if indeed the clinical difference due to the melatonin intervention causes a 15% absolute change (say from 20% to 5%) then with a sample size of 640

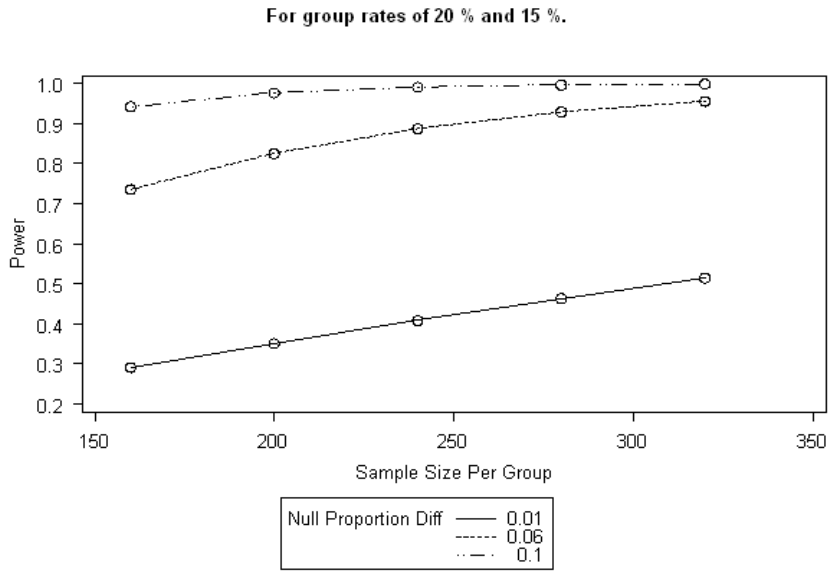
we essentially have 100% power to detect this difference i.e. this difference will be incredibly unlikely to be missed due to chance.

Figure 2: For group rates of 20 % and 5 %, power versus sample size per group for different null rate differences.



In the third and final graph (Figure 3) if we see an event rate of 20% which is also possible in our population and there is a drop of only 5% we are actually powered to nearly 90% with a population of 640.

Figure 3: For group rates of 20 % and 15 %, power versus sample size per group for different null rate differences.



For the phase II sub-study, we are interested in exploring potential mechanisms through which melatonin may be exerting activity. An exploratory pilot analysis of 80 subjects will provide information on what is occurring physiologically and is powered to see a 30% significant shift in NK cell functional activity. In addition, with 40 patients in each group, the expected number with recurrence or mortality is expected to be approximately 16 subjects, allowing for comparison of the NK cell function and biologic markers amongst this subgroup, compared to those without recurrence for both the melatonin and the control groups.

Although sample size calculation represent only rough estimates of the true sample required [79], confidence intervals around the primary endpoint will provide strong inferences into how well powered the trial is. It should be noted that our proposed sample is much larger than any of the previously conducted melatonin/cancer trials for which a statistically significant reduction in mortality was found after only one year. Given the sample size we will accrue, we confidently anticipate that the trial is well powered to address the primary objective without undue use of time and resources. The addition of the mechanistic substudy and trial extension will greatly extend the impact of the trial and potential for strong results to lead to policy shifts and a change in pattern of clinical practice leading to better patient care.

8. Statistical Analysis

The primary analysis will be performed using an 'intention to treat' analysis. Using this approach, patients will be included in the analysis according to the group to which they were randomized regardless of cross-over or compliance. Baseline characteristics of patients in the two treatment arms will be assessed using frequency distributions and univariate descriptive statistics including measures of central tendency and dispersion. As a complementary approach,

we will also conduct a treatment received as 'per protocol' analysis of the primary outcome measures. Baseline characteristics in the two treatment arms will be compared using univariate descriptive statistics.

The primary analysis of the composite outcome of two-year cancer recurrence and mortality will be conducted using an unadjusted chi-square test comparing the proportion of events in each of the two treatment groups. This will be translated into a relative risk (RR), relative risk reduction (RRR), absolute relative risk (ARR), and number needed to treat (NNT). In addition, a logistic regression procedure will be employed to adjust exacerbation proportions using covariates that are either clinically important prognostic risk factors or unbalanced at baseline.

Phase II substudy analysis

Cytokine array

To determine the impact of melatonin on patient cytokine levels, a cytokine array will be generated for each patient. Briefly, patients will be divided into two cohorts; those receiving melatonin and those receiving placebo treatment. Following data collection, cytokine expression measurements will be log transformed, and a KS-test will verify normality within each dataset. If confirmed, a one-way anova will assess if any cytokines are significantly affected, followed by a t-test corrected for multiple hypothesis testing (bonferonni) to identify which are significantly affected by melatonin treatment. If failed, a Kruskal–Wallis one-way anova will assess if any cytokines are significantly affected, followed by a Wilcoxon signed-rank test corrected for multiple hypothesis testing (bonferonni) to identify which are significantly affected by melatonin treatment.

Flow cytometry

To determine the impact of melatonin on NK cell markers, flow cytometry will be performed. Statistical analysis will be similar to that implemented in the cytokine analysis. Following confirmation of normality (KS-Test), a one-way anova will assess if any markers are significantly affected followed by a t-test corrected for multiple hypothesis testing (bonferonni) to identify which are significantly affected by melatonin treatment. If normality is not identified, a Kruskal–Wallis one-way anova will be performed to assess if any markers are significantly affected, followed by a Wilcoxon signed-rank test corrected for multiple hypothesis testing (bonferonni) to identify which are significantly affected by melatonin treatment.

NK cell cytotoxicity assay

To characterize whether NK cells become activated following melatonin treatment, chromium release assays will be performed for both cohorts; those receiving melatonin (M) and those receiving placebo (P) treatment. Following fitting both cohorts to a model representative of each distribution, statistical analysis will be performed to assess if melatonin had a significant impact on NK cell impairment. This statistical analysis relies on the logarithmic error ratios (LER) for each treatment where $LER = \log(SSR_M/SSR_P)$, SSR is the sum of squared residuals and M/P are the models respective of each cohort. When the fits to M/P differ from one another, it is anticipated that the LER will be significantly different between both datasets. The subsequent probability associated with the null hypothesis that two fits perform equally well will be calculated by cross-validation. Briefly, 2 patients from each cohort are randomly selected to generate a training and validation datasets of equal size for each treatment. Following fitting the

data of each cohort using the training dataset, two LERs will be calculated using the validation dataset for each cohort. By repeating this process 1000 times, we will obtain a distribution of LER for each cohort. If LERs associated with the fits to M/P are normally distributed, a t-test will be performed to assess the likelihood that the killing assays are identical to one another.

In order to determine the reliability of the questionnaires evaluating the secondary outcomes, we will conduct an Analysis of Co-variance as well as determine Cronbach's alpha.

9. Ethics

The study proposal and patient consent form has been approved by the Research Ethics Boards at the Canadian College of Naturopathic Medicine, The Ottawa Hospital, participating multicenter sites, and by The Natural Health Products Directorate. Ethics Committees and the Data and Safety Management Board are provided with annual progress reports.

10. Study Timetable

Recruitment for this trial will take a total of eight years to complete, with an expected end date of January 2015. Following the closing of recruitment primary outcomes will be assessed. The study will be closed and all data finalized, two years following enrollment of the last participant. Dissemination will take place through publication in a high impact journal, through conference presentations at national and international conferences, and to lung cancer patients through outreach to patient advocate groups and societies.

11. Team of Investigators

Principal investigator:
Dugald MR Seely ND, MSc is a naturopathic doctor and cancer researcher with experience in conducting clinical trials as well as being a professor of research methodology at The Canadian College of Naturopathic Medicine (CCNM). Dr Seely is the Director of Research at CCNM and has devoted much time to both epidemiological and preclinical research on melatonin for the treatment of cancer. Dr Seely is highly active in the field of complementary and alternative medicine for cancer and has presented and published extensively in this area. Dr Seely will coordinate all aspects of the study to ensure that the execution of the clinical trial is properly conducted during all phases.

Clinical Site Principal Investigator and Trial Coordinating Center Lead/TOH site QI:
Andrew JE Seely MD, PhD is a thoracic surgeon and intensivist at The Ottawa Hospital (TOH), is an Associate Scientist at the Ottawa Hospital Research Institute (OHRI), and holds a CIHR New Investigator award. Dr A Seely is a critical care physician and a surgeon, with basic science research training, whom is actively involved in research applying complex systems science to patient care. Dr A Seely will be active during all phases of the study including recruitment, assessment, and analysis. As PI for the coordinating center, in conjunction with Dr D Seely, Dr A Seely is responsible for overseeing the conduct of the trial at the multicenter sites.

Dr. A Seely is also the Qualified Investigator for the site at TOH and therefore is responsible for the conduct of the trial at the site.

TOH Co-investigators – Thoracic Surgeons:
Donna E Maziak MD, MSc is a thoracic surgeon with a Master's in Clinical Epidemiology, with extensive research experience in prospective clinical research. Specific experience includes helping coordinate a CIHR funded large multi-center randomized controlled clinical trial regarding the use of Positron Enhanced Tomography (PET) imaging in the treatment of lung cancer.

R. Sudhir Sundaresan MD is a thoracic surgeon with extensive training and experience in basic science, with an international reputation for integrity and leadership. Dr Sundaresan is Professor and Chief of the Division of Thoracic Surgery.

Farid M. Shamji MD is a senior thoracic surgeon, having single-handedly built the Division of Thoracic Surgery at the University of Ottawa since 1983. He is renowned for his teaching skills, surgical leadership and vision; and is the President of the Canadian Association of Thoracic Surgeons.

Co-investigators – Oncologists:
Scott A Laurie MD is a medical oncologist specializing in lung cancer at The Ottawa Hospital Regional Cancer Centre, has extensive experience in clinical research and will be active in recruitment and assessment of patients during all phases of the trial.

Rob M McRae MD is a radiation oncologist specializing in lung cancer at The Ottawa Hospital Regional Cancer Centre, has extensive experience in clinical research and will be active in recruitment and assessment of patients during all phases of the trial. Drs McRae and Laurie will be the primary physicians for all lung cancer patients enrolled in the clinical trial and will work with the Research Coordinator to ensure that all eligible patients are approached for consent and followed according to the trial protocol.

Co-investigators – Epidemiologists:
Dean A Fergusson MHA, PhD is an epidemiologist and experienced trialist, and will be responsible for data management, methodological rigor, and in all aspects of data analysis.

Clinical Site/Coordinating Center:
In conjunction with a highly effective inter-disciplinary team of respirologists, radiologists and oncologists, the five thoracic surgeons who comprise the Division of Thoracic Surgery at the University of Ottawa at The Ottawa Hospital currently treat more lung cancer patients than in any other center in Ontario, and perform the largest number of pulmonary resections than any other group. Working in a group practice, the Division is known for its cohesive and comprehensive care of lung cancer. The Division is a leading center of Thoracic Surgery in Canada, with numerous research and administrative contributions recognized nationally and internationally.

12. Feasibility

The combination of expertise in lung cancer treatment, natural health products, cancer research, and trial methodology in an ideal clinical setting puts our team of investigators in the position to fully address the objectives laid out. The assembled team of investigators includes all the people needed to successfully complete the trial including a naturopathic doctor, four thoracic surgeons, two epidemiologists, a medical oncologist, and a radiation oncologist. With the support of the Lotte and John Hecht Foundation, this seminal partnership between The Canadian College of Naturopathic Medicine and The Ottawa Hospital have the combined resources to effectively execute and complete this very important clinical trial.

The specific benefits of partnering with The Ottawa Hospital are considerable. Primarily, the Department of Thoracic Surgeons sees one of the highest volumes of lung cancer patients eligible for surgical resection in all of Canada. Affiliation with this group allows the accrual of enough patients in a single center, thereby substantially reducing the associated costs and complexity of the trial. With regards to this, The Ottawa Hospital Research Institute (OHRI) has recognized that this trial will not be benefiting from the usual support obtained from the pharmaceutical industry. Based on this and the novelty of the trial, the OHRI has graciously agreed not to add its usual 25% overhead costs. A further cost reduction has been achieved by securing the commitment from SISU Inc. (the supplier that provided the melatonin/placebo capsules for the first phase of the trial) to provide both the melatonin and placebo free of cost. This contribution has a market value of approximately $ 50,000. It is essential to note that this donation is made with no company input or direction on trial design or with any limitations or requirements for post-trial publication and dissemination.

13. Dissemination/Knowledge Transfer

Given the important and innovative aspects of this study, widespread dissemination of the trial results will be performed. This will include scientific presentation to both alternative and traditional medical societies within Canada and internationally. Presentations will be accompanied by published abstracts. However, the principal mechanism for knowledge transfer will be publication. We will target the most reputable clinical journals for publication due to the potential impact of this investigation (e.g. New England Journal of Medicine, Lancet, or British Medical Journal). Lastly, presentation of the results will be performed locally in Ottawa and Toronto through Grand Rounds and other educational round opportunities. We will additionally translate our findings for cancer patients and lay persons through presentations primarily at lung cancer support societies.

14. Relevance and Impact

Lung cancer kills far more men and women than any other cancer. Despite advances in surgical therapy and decades of research regarding chemotherapy, recurrence rates leading to mortality remain unacceptably high. The potential for relatively benign natural health products to improve outcomes remains an attractive therapeutic modality too often ignored by the medical profession. Melatonin has strong but not definitive evidence supporting its use as an anti-neoplastic agent. A properly performed large randomized clinical trial is essential to evaluate the clinical value of this therapy. If proven effective, this trial will be the most effective way to allow lung cancer

patients to benefit from melatonin, as well as demonstrating the potential value of natural health products within mainstream health care in general. Conversely, if proven ineffective, the large number of patients currently taking melatonin with inadequate evidence to support its use will have good evidence to discontinue its use.

Although results from small trials testing the benefits of adjuvant melatonin in NSCLC are encouraging, confirmation from an independently based trial is necessary. The trial proposed is properly powered and rigorously designed so as to answer the question of the effectiveness of melatonin in the prevention of lung cancer recurrence and mortality at 2-years. The fact that lung cancer is such a deadly disease with truly inadequate conventional therapies underscores the importance of testing novel treatment regimes. Melatonin is an example of a natural health product with extensive and mostly positive research on its potential anticancer effects. What is interesting about melatonin is that it has no traction as a treatment protocol in conventional settings as it is not a patented pharmaceutical drug. By conducting a well-powered, rigorous trial, we aim to address the question of the effectiveness of melatonin head on and provide conclusive answers for this potentially lifesaving adjuvant therapy.

In addition to testing the therapy itself, the execution of a clinical trial like this in a well-known research hospital will have the important net effect of introducing a therapeutic agent that has not sprung from the pharmaceutical industry. Instead, a conventional medical institution will be collaborating with CCNM, an institution dedicated to the training and research of complementary and alternative medicines, towards the research of a natural health product.

The public is adopting natural medicines as a regular part of their personal health choices. According to Health Canada's website, a recent survey showed that 71% of Canadians regularly take vitamins and minerals, herbal products, homeopathic medicines and the like - products used in complementary and alternative medicine that have come to be known as natural health products (NHPs). With such a large percentage of the population interested in using NHPs to improve their health, there has been a serious deficiency in the number of scientifically controlled studies to prove the benefits and harms of these products. Since NHPs are often not patentable, there is little funding from traditional pharmaceutical or other for-profit agencies to support these scientific studies. With only anecdotal evidence to inform decision making, we know that many patients are being harmed by the improper use of NHPs and many more are not receiving the benefits.

This study is unique. By working in partnership with an academic health science centre, with well-established and qualified clinical researchers, by applying the same rigid standards to this research as to any other clinical pharmaceutical research, the investigators are taking a step forward in forging a new method of conducting critically needed research into NHPs. This kind of research is critical because it meets the needs of the medical community for rigorous, scientific methodology, with consideration given to the differences in studying NHPs. The impact of these partnerships in future endeavours cannot be understated. Many organizations and investigators will be monitoring this study to learn and develop their own research and partnerships. The significance of this study is not limited to its importance for the patient with lung cancer, but it will have a wide impact on the credibility of scientifically funding and testing NHPs throughout Canada.

15. Detection of Recurrences (Discrete Student Research Project)

The question has been raised as to how the recurrence of lung cancer occurs and is best detected. This question lends itself very nicely to a discrete student research project that can further look at the recurrences that have been identified in the AMPLCaRe research project.

The first step is to conduct a literature review to summarize the current research in the area and to identify the variables to be collected in the current project (e.g., time to recurrence, were symptoms present, modality of identification, etc.). These variables will then be extracted from the charts of the patients who recurred at this site (approximately 40 at this point). This project will be conducted at the Ottawa Hospital only.

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