# STUDY PROTOCOL

## Pre-Treatment of Highly Suspicious Pigmented Skin Lesions with Interleukin-2

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## Lay Summary

Skin cancer is the world's most common form of cancer – with more diagnoses annually than breast, prostate, lung, and colon cancers combined. Melanoma, the most aggressive form of skin cancer, has killed an estimated 1250 Canadians in 2017 alone. When discovered early, melanoma can usually be cured with surgery alone, but once it metastasizes therapeutic options are limited. Up until 2011, only two therapies were approved by the US food and drug administration (FDA) for the treatment of advanced metastatic melanoma (MM) – Dacarbazine (DTIC) and systemic Interleukin-2 (IL-2). In the past 10 years of variety of immunological agents have been approved for the treatment of MM including direct injection (intralesional) of IL-2 into tumors.

IL-2 naturally occurs in the immune system and has been used as a cancer immunotherapy for almost 40 years. Systemically administered melanoma immunotherapies, such as IL-2, show promise but are at high doses have morbid toxicity profiles and are costly. By administering IL-2 intralesionally via injection, local efficacy and toxicity profiles are improved, compared to systemic administration, as lower doses of IL-2 are needed and are administered directly into the lesion. IL-2 treats MM by engaging the immune system. One of the potential benefits theoretically from this therapy is the creation of lasting tumor immunity that could protect the patient from further development of metastatic disease. The hallmark of an effective immune response is the presence of a robust population of immune cells, so called tumor infiltration lymphocytes (TILs), identified histologically in surgically removed tumors.

When a patient is identified having a highly suspicious pigmented lesion, the diagnosis of melanoma is confirmed with an excisional biopsy (a biopsy that grossly includes a narrow rim of uninvolved tissue), part of the normal standard of care. When present, demonstration of a robust population of TILs in the biopsied specimen is a strong indicator of a natural immune response. We aim to assess the utility of treating highly suspicious pre-metastatic lesions in a randomized, placebo-controlled, trial of intralesional IL-2. We hypothesize that a "proactive" pre-treatment strategy with IL-2 will boost local immune response to suspicious lesions by stimulating a robust TIL response that could ultimately prevent distant metastasis. We believe this to be the future of melanoma therapy.

We aim to include 20 local participants over the next 24 months in a randomized, placebo-controlled, trial of intralesional IL-2 to assess the utility of treating highly suspicious pre-metastatic lesions. Patient consent will be obtained using an NS Health Authority, REB-approved SOP. All patients will have lesions biopsied following standard surgical practice techniques, and will provide blood and urine for analysis. Tissue samples will be assessed for immune system activity, and blood and urine will undergo proteomic and metabolomic analysis.

## Background Information:

Skin cancer is by far the world's most common form of cancer – with more diagnoses annually than breast, prostate, lung, and colon cancers combined.[1] Every year in Canada over 80,000 cases of skin cancer are diagnosed, over 7,200 of which are Melanoma, the most deadly form of skin cancer.[2] It is estimated that in 2017, over 1250 Canadians have died of Melanoma.[2]

The incidence of melanoma in Canada is on the rise in both men and women. In women the incidence of melanoma has increased by 2.8% between 2001 and 2010, an annual percentage

change surpassed only by thyroid cancer.[2] As mortality rates for almost all other forms of cancer decrease – the age-standardized mortality rate of melanoma is on the rise. Melanoma is the number one cancer killer of women aged 25 to 30.[1]

As a whole, 5-year age-standardized survival rates for melanoma have been reported as high as 88%.[2] When discovered early, melanoma can usually be cured with surgery alone, but once it metastasizes therapeutic options are limited. 5-15% of patients with melanoma will progress to stage IV MM.[3,4] MM is one of the most aggressive malignancies, with 5-year survival rates of advanced disease between 5-23%, depending on the location of tumor dissemination.[5,6] Although several new therapeutic interventions for metastatic melanoma are undergoing clinical trials, for the foreseeable future MM is a disease characterized by a worrying rate of mortality, and treatments of questionable efficacy.[5,7]

*Given the paucity of effective therapeutic interventions for MM, successful strategies in treating melanoma will include therapies or therapeutic adjuvants which limit, or altogether prevent metastases.*

## Current Treatments:

*Limited therapeutic Options for Metastatic Melanoma*

Cure rates for melanoma are high when the disease is discovered before it has spread from its primary location; however, metastasis frequently occurs, presenting a clinical challenge that has frustrated physicians and researchers alike for decades. Up until 2011, only two therapies were approved by the US food and drug administration (FDA) for the treatment of advanced (stage III or stage IV) MM. DTIC, the only chemotherapeutic licensed to treat MM was approved in 1975, has limited therapeutic utility. Patients on DTIC can expect a one-in-eight chance of having tumors shrink.[8] Systemic therapy utilizing high-dose IL-2 was approved by the FDA in 1998. IL-2 is a naturally occurring glycoprotein secreted by T cells to mediate cellular immune response, and has been used as a cancer immunotherapy for almost 40 years.[9] IL-2 mediates bystander activation and proliferation of CD4+ T-cell, CD8+ T-cell lymphocytes and to a lesser extent, NK cells.[9] Although 4% of patients were cured using this immunological therapy, the side effects of systemic administration are significant and are fatal in 2% of patients.[10] Even with these treatments, the outcome for patients with distant metastases is bleak, with median survival of 6 to 10 months and less than 5% of patients surviving for more than 5 years.[6]

*Melanoma Immunotherapies*

Indeed, immune control of melanoma is achievable in specific circumstances – elucidation of the molecular identity of several antigens that are recognized by the immune system of melanoma patients has led to the discovery of pathways affecting tumor immunity at a cellular and molecular level.[11–13] Still MM has proven to be excessively difficult to treat, and the development of novel immunologically-mediated therapeutics has been slow. Many adjuvant vaccine trials in melanoma have been conducted including GMK vaccine and granulocyte-monocyte colony-stimulating factor (GM-CSF), but these have been largely unsuccesful.[14,15] Similarly, trials assessing the treatment of MM with immune stimulants such as Bacillus Calmette–Guerin (BCG), Corynebacterium parvum and levamisole have yielded mixed inconsistent responses or been ineffective and even harmful to patients.[16]

Significant inroads have been made in recent years with the development of novel immunotherapies for the treatment of MM. In 2011 the FDA approved the use of monoclonal antibody ipilimumab as a checkpoint immunotherapy for the treatment of advanced MM.[17] Ongoing studies are assessing whether adjuvant therapies such as chemotherapy or various immunologic therapies may improve on the antitumor effects achieved with ipilimumab.[7] Multiple systemic antibody-mediated immunotherapies for advanced MM – including nivolumab and pembrolizumab – are current under development, and have shown significant advances;[18] However response to these novel therapeutics have been tempered as these therapies are associated with serious (sometimes fatal) immune-mediated side effects and prohibitive pricing.[19]

## Intralesional Therapies

The serious toxicity profile associated with systemic immunotherapies can be altogether avoided by treating instead with intralesional injections. Studies have shown lowers rates of toxicity are associated with intralesional injections when compared with systemic administration; furthermore, by delivering an increased concentration of the drug at the site of action, increased rates of efficacy are observed.[20] Intralesional IL-2 is associated with modest flulike symptoms alone – a vast improvement on the toxicity profile associated with systemic administration of IL-2.[10,21] Intralesional injections of IL-2 have an added bonus of causing a so-called "bystander effect", whereby cancerous cells that are not immediately adjacent to the local injection site, also are effectively treated through development of an adaptive regional immune response.[20]

## "Proactive" vs "Reactive" Treatment: The Future of Melanoma Therapy

IL-2 has been effectively used to treat MM when administered intralesionally.[21] This mode of treatment using IL-2 is being effectively delivered by Dr. Carman Giacomantonio to treat patients with advanced cutaneous MM at the QEII HSC, NSHA. IL-2 used in this capacity is a "reactive" treatment to MM. Herein we propose the use of IL-2 as a "proactive" treatment to pre-metastatic melanoma.

We plan to treat patients with highly suspicious lesions – as diagnosed by qualified dermatologists – with intralesional IL-2 in an effort to generate an adaptive immune response with activation and proliferation of CD8+ T-cell effector lymphocytes and immune sensitized CD8+ T memory cells to address the potential risk of subsequent melanoma metastasis.[9] Moreover, after treatment with IL-2, the proliferation of CD8+ T-memory lymphocytes may allow cells of immune surveillance to mount an immune response to new, *de novo* pre-cancerous and/or cancerous melanoma cells.

*With 1250 estimated deaths in Canada in 2017 due to metastatic melanoma, finding preventative treatments is crucial. We believe that a "Proactive" pre-treatment with IL-2 is the future of melanoma therapy.*

## Hypothesis:

*Hypothesis*: Pre-treatment with IL-2 in patients with highly suspicious pigmented lesions will reduce the incidence of metastatic melanoma compared to the PBS placebo group by initiating an adaptive immune response to native melanoma protein and peptide.
*Major Aims*:

1) To conduct a pilot study to assess the number of patients needed to analyze in order to achieve a statistically significant differentiation between treatment and control outcomes in study measures including tumor infiltrating lymphocytes (TILs) and circulating immunomodulators.
2) To assess the efficacy of intralesional pre-treatment of IL-2 on highly suspicious lesions in generating an adaptive immune response and preventing melanoma metastasis.
3) Sub-study – To assess if there a difference in specific immune modulators in patients receiving IL-2 pre-treatment compared to patients receiving a placebo.

**Subject Selection:**

Qualified dermatologists will identify and approach potential participants to recruit them to become part of the study. The participant population will include patients characterized by: nodular/polypoid features, bleeding/ulcerated lesions, excluding face and vulvo-genital lesions; and who are not: currently immunocompromized, on immuno-therapy for other diagnosis, have known inflammatory or autoimmune diseases or are otherwise incapacitated, between the ages of 18 and 80 years of age. We aim to include a minimum of 20 (up to 60) local participants over the next 24 months. Patients will be randomized and will receive a subcutaneous injection of either IL-2 treatment (treatment group) or the placebo PBS treatment (placebo group).

**Participant consent:**

Consent will be obtained using an approved NS Health Authority REB protocol. It will be clear to the patient that it is important that they tell their surgeon about any drugs or medicines they are taking or wish to take and that they must also tell their surgeon if they are having any adverse effects. Patients will be allowed to withdraw from the study protocol at any time. It will also be clear that if they withdraw consent, the information about them and their donated tumor tissue that was collected before they left the study will still be used. No new information about them will be collected (and no further testing of donated tumor tissue, blood or urine will be done) without permission.

**Research Plan:**

Clinical Research Plan: Is intralesional pre-treatment of IL-2 in highly suspicious pigmented lesions effective in preventing future melanoma lesion spread and metastasis?

This study is primarily designed to determine if tumor specific immunity can be generated in patients with highly suspicious pigmented lesions in response to intralesional IL-2, and whether

that immunity can confer resistance to melanoma metastasis. Patients will be identified by qualified dermatologists and interviews will be held at the surgery clinic (4th floor Dickson Center) QEII HSC, NSHA.

The standard wait time from consultation to surgical biopsy is up to 4 weeks. We will ensure that patients are seen immediately upon notification from participating dermatologist and all research components of the study are completed within the normally anticipated wait time. Through utilization of this standard wait time, we can potentially affect an immune response using intralesional IL-2, that otherwise could not be achieved after the biopsy is completed. Given that the study is conducted within the normal wait time, it doesn't deviate from the normal standard of care. Following our protocol, patients will receive intralesional injections on Day 1(Week 1 Visit) and Day 8 (Week 2 Visit), and excisional biopsies will be performed on Day 15 (Week 3 Visit), well within the accepted wait time from consultation to biopsy. The intralesional injections and collection of biospecimens beyond the biopsy deviate from but does not delay the normal standard of care. Two additional visits are required in addition to the initial consultation.

This study is a randomized, controlled, double-blind study. Patients with highly suspicious pigmented lesions will be randomly assigned by an algorithm to one of two groups: 1) treatment group patients will be treated intralesionally with IL-2 (Proleukin (Aldesleukin), Novartis Pharmaceuticals Canada Inc.) at a dose of 500,000 International Units (IU) (0.1ml) for 2 treatment cycles 1 week apart (Day 1 and Day 8) Figure 1; 2) control group patients will be treated intralesionally with sterilized sodium chloride (0.9%, m/v) (0.1ml) Figure 1. Randomization will be done at the pharmacy for each patient (generated by a Random Block algorithm). IL-2 (Proleukin) will be obtained from the Provincial Drug Distribution Program by the pharmacy, paid for by Dr. Carman Giacomantonio's research services account (please see budget attached). The pharmacy will prepare either IL-2 treatment (supplied as 1 x 0.8ml (4 million units) or injectable placebo (0.9% sodium chloride) supplied as 0.8ml). The syringes will be prepared by following aseptic technique in a biohazard hood. A 22-million unit vial of IL-2 will be reconstituted with 1.2ml of sterile water for injection and mixed gently. After drawing up 3.2ml of 5% dextrose in water and transferring to the IL-2 vial, the resulting concentration will be 5 million units/ml. 0.8ml of the IL-2 solution will then be drawn into a 3ml syringe. Expiry time of the reconstituted solutions is 48 hours at 4C. The pharmacy will assign a codified ID which will be provided along with the syringe with the patient name labeled as "Interleukin-2/Placebo" (thus blinding study to clinician and patient). As a pilot study, the principle of the design is to test the feasibility of proceeding to a larger and more expensive trial following the methodology and protocol proposed. As such there will be a minimum of 20 (10 treatment, 10 placebo) participants (up to a maximum of 60). It is estimated that statistical significance will be reached with 20 patients, however it is not reached after 20 patients, an additional 10 (5 treatment, 5 control) patients will be enrolled. If significance is still not reached after 30 patients, an additional 10 patients will be enrolled and so on up to a maximum of 60 patients. If after 60 patients, significance is not achieved then further recruitment under the current protocol would not be logical and therefore the methodology would need to be revised or study discontinued.

On Day 1 (first treatment) and 15 (excisional biopsy), all patients will have blood (4 vials) and urine (25-50 ml) samples taken. Local reactions to injections will be monitored for non-specific signs such as bleeding, arythema, infection, or irritation. We don't anticipate specific changes in the pigmented lesion related to the injection in this short time period, however all changes will be noted.

On Day 15, following the second intralesional injection an excisional biopsy will be performed following standard surgical techniques, as follows: ellipse of skin encompassing the pigmented lesion extending into the subcutaneous fat will be performed to achieve a grossly clear but narrow margin of excision. This small defect will be closed primarily with interrupted sutures. The biopsied specimen will be subsequently evaluated using standard histological techniques to confirm diagnoses, assess margin status (clear or involved) and depth of lesion invasion. The depth of lesion invasion will dictate the extent of subsequent surgical excisions and margin selection according to standard National Comprehensive Cancer Network (NCCN) guidelines. Pre-biopsy intralesional injections of 0.1ml into the lesion will not alter dimensions or architecture of the lesion or impact the extent the subsequent biopsy required. Lesions that are felt to be too large for closure without tissue manipulation or creation of flaps, will be biopsied using a punch biopsy sampling technique whereby 4mm diameter discs of tissue from representative areas of the lesion will be taken to confirm the diagnosis and the depth of invasion. The depth of invasion will dictate the extent and complexity of subsequent surgery required to remove the lesion and achieve clear margins according to NCCN guidelines. The biopsied specimen will be processed as follows: using a 22G needle a fine needle aspiration biopsy will be performed (the needle will be passed through the center of the lesion two times) for RNA analysis. The remainder of the tissue will be sent to pathology for standard histological assessment. The pathologist will also report the extent of observed tumor infiltrating lymphocytes (TILs) associated with treatment compared to control injections. Blood and urine will be assessed using metabalomic and proteomic methods to assess systemic immune response to treatment.

All blood, urine, and lesion biopsy samples will be labeled with a codified number (will not contain any patient identifying information) and will be immediately transported to Dr. Carman Giacomantonio laboratory (Sir Charles Tupper Medical Building, 11F11) at Dalhousie University for storage. Fine needle aspiration of excised samples will be assessed for tumor genetic and epigeneitic profile (Figure 2). Blood and urine will be assessed for proteomic and metabolomic profiles, respectively (Figure 2).

A database containing patient IDs and codified numbers will be restricted to the office of Dr. Giancomantonio (11th floor, VG). This is a locked office, and the data will only be accessible to the investigators named on this application. This database for will contain patient IDs, and info on disease status, treatment/control, follow up visits, and sample analysis – only info pertinent to the outcome measures. All sample data will be assessed using codified descriptors and will contain no patient ID info. Once the RNA, proteomic, metabolomic and pathology data has been analyzed, unmasking treatment and control groups will be conducted at the VG office of Dr. Giacomantonio, by the named investigators. If the data reaches statistically significance, the study will not accept any more patients, if not, a further 10 patients will be incorporated – to a maximum of 60 patients. Any publications of study results will be completed devoid of any information that could be used to identify patients included in the study.

All study participants will receive biannual assessments for 5 years after the initial intervention to assess disease progression, or the development of new melanoma, to compare between both treatment and control groups. There is no standardize test or measurable biomarker to assess established or lasting immunity. This aspect of the study is identical to the patient assessment conducted as per standard of care for melanoma patients. Again, any publications of study results will be completed devoid of any information that could be used to identify patients included in the study.

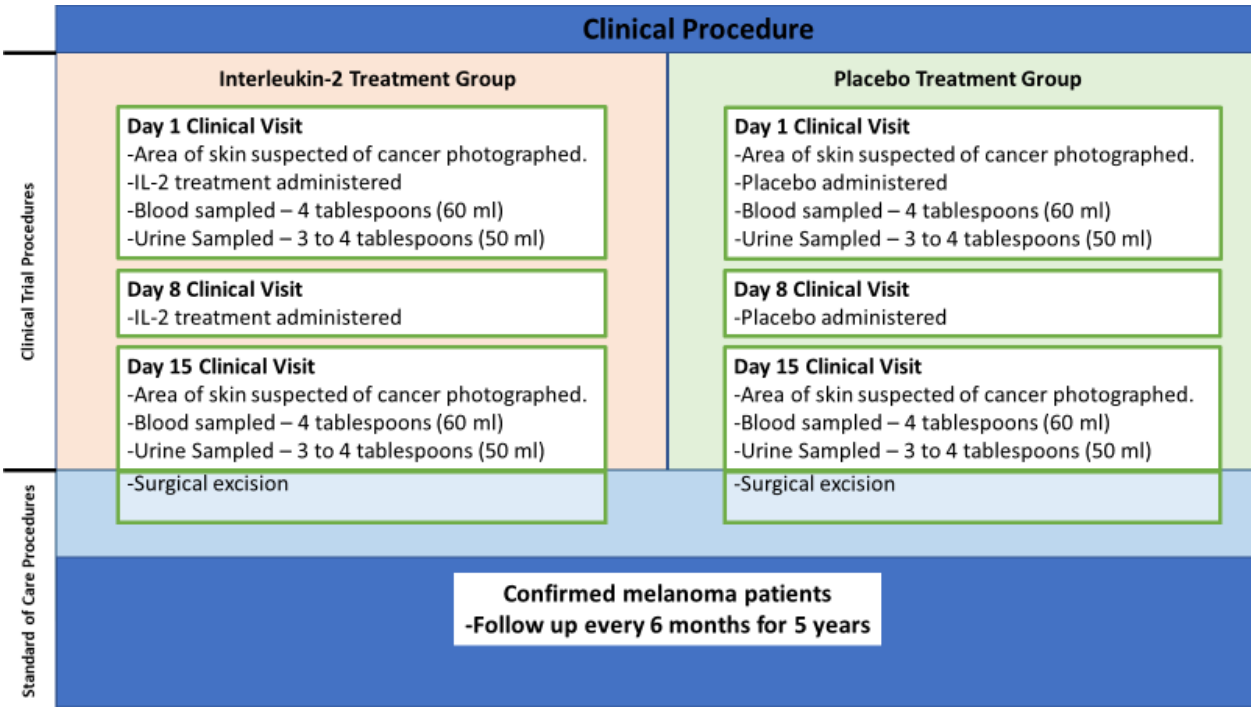


**Figure 1** – Clinical procedure

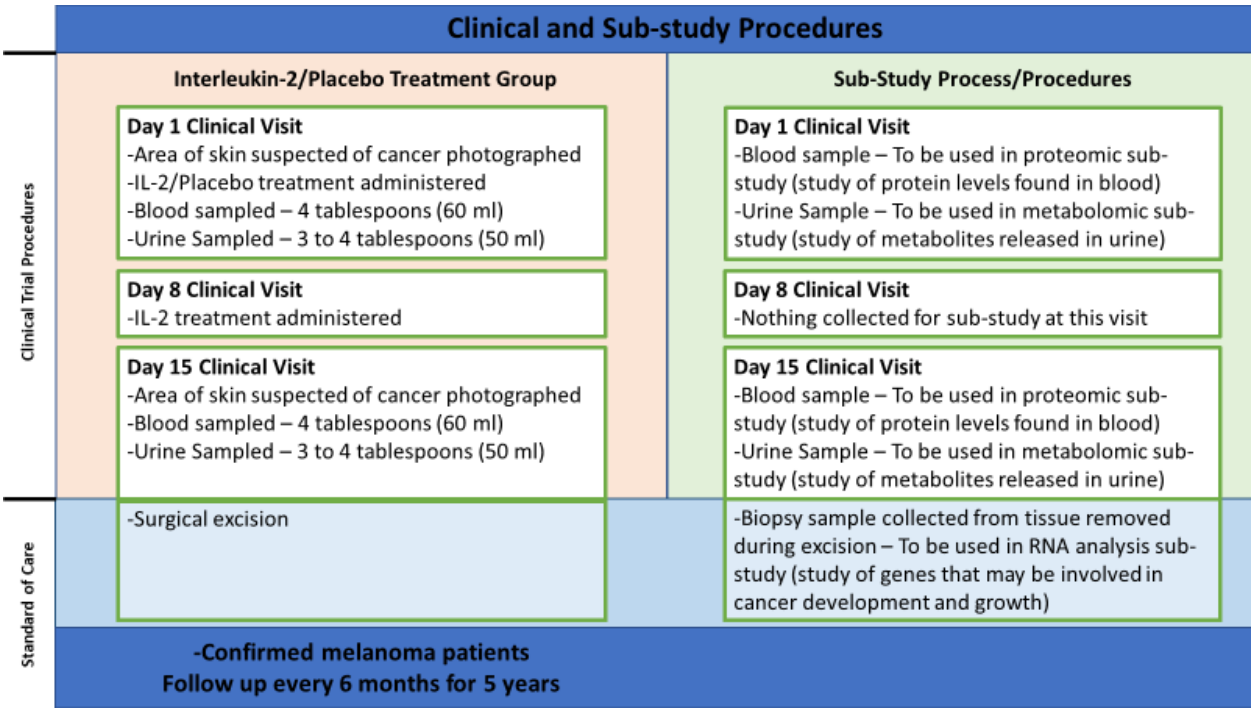


**Figure 2** Clinical and Sub-study Procedures

## Analysis of Data

## Outcome measure/study objectives:

Assessment of Number of Patients Needed to Obtain Significance [**Time Frame: 1 year**] – Data will be analyzed in order to achieve a statistically significant differentiation between treatment and control outcomes in study measures including tumor infiltrating lymphocytes (TILs) and circulating immunomodulators.

Assessment of Metastasis [**Time Frame: 5 years**] - All patients will receive assessments every 4 months for 2 years and then biannual assessments for years 3-5 after the initial intervention to assess disease metastasis in treatment and control groups. Both number of new metastases (integer value) and thickness (mm) will be measured as a part of this assessment.

Sub-Study Outcome Measure:

Assessment of RNA genetic profile **[Time Frame: 5 years]** - RNA analysis of excised tissue will be compared to unaffected patient tissue obtained from the clear margins of the excisional biopsy to assess genetic changes resulting from the melanoma and treatment/placebo injections. RNA analysis will occur at Dr. Giacomantonio's laboratory (Sir Charles Tupper Medical Building, 11F11) at Dalhousie University.

Assessment of Systemic Immune Response: Proteomic Analysis [**Time Frame: 5 years**] - Proteomic analysis will be conducted on blood samples to assess systemic immune response to both treatment and control groups. Serum collected from patient blood samples will be used for proteomic analysis to assess protein expression, including circulating immunomodulators (cytokines and chemokines) before, and after, treatment. Proteomic analysis will occur at Dalhousie's Proteomics and Mass Spectrometric Core Facility located in the Life Sciences Research Institute. This study may serve to help develop diagnostic protocols and methods of assessing response to treatments.

Assessment of Systemic Immune Response: Metabolomic Analysis [**Time Frame: 5 years**] - Metabolomic analysis will be conducted on urine samples to assess systemic immune response to both treatment and control groups. All tumor and tissue produce by products in waste that are excreted by the kidneys. Urine samples can be evaluated using techniques, such as Mass Spectrometric, to determine if biological compounds can be identified in association with the presence of a malignant process that would not be produced by normal tissue. Metabolome analysis will occur at Dalhousie's Proteomics and Mass Spectrometric Core Facility located in the Life Sciences Research Institute. This study may serve to help develop diagnostic protocols and methods of assessing response to treatments.

**How will the data be analyzed:**

All study practices and statistical methods are based on the International Conference on Harmonization (IHC) document "Statistical Principles for Clinical Trials." Data will be summarized by treatment group. Baseline characteristic, safety outputs and a total overall column will be included to summarize all subjects. For all baseline, demographic, safety and efficacy outputs data will be summarized by treatment group.

In summary tables of continuous variables, the minimum and maximum statistics, the arithmetic mean and median, the 95% confidence interval, standard deviation, and standard error will be presented will to the same number of decimal places as the original data. In summary tables of categorical variables, counts and percentages will be used. The denominator for each percentage will be the number of subjects within the population treatment group unless otherwise specified. All hypothesis testing will be carried out at the 5% (2-sided) significance level unless otherwise specified. P-values will be rounded to three decimal places. P-values less than 0.001 will be reported as <0.001 in tables.

The treatment label for all tables, listings and figures will be:

| **Treatment** | **Label** |
|---|---|
| 2 treatment cycles of 500,000 IU of IL-2 in 0.1 mL | IL-2 Treatment |
| 0.1 mL of sterile saline (0.9% m/v) | Placebo |
| All Treatments | Total |

Where that any of the statistical methods described herein prove unsuitable during analysis, more appropriate methods will be used. All changes in methodology will be documented in the clinical study report. Additional ad-hoc analyses may be conducted as deemed suitable.

Subject inclusion/exclusion criteria will be determined at baseline visit, and subjects who do not meet all criteria will not be entered into the study. Those subjects deemed eligible to participate will be allocated a 3-digit number at randomization prior to the initial treatment. If a subject is discontinued at any time after entering the study, the Investigator will ensure this does not affect the patient's standard of care. At the patients request all unused biological samples (blood, urine, and core biopsies) will be immediately destroyed. The reasons for withdrawal will be recorded on the CRF and will be included in the final report. Failure to complete both (2) treatment cycles will result in patient removal from the trial.

Data resulting from our RNA, metabolomic and proteomic studies will be analyzed using specific statistical software available to us at Dalhousie University, including Bio-Rad qPCR machine and CFX manager statistical software for RNA analysis, and liquid chromatography-tandem mass spectrometry (LC-MS/MS) systems (QTRAP5500 by ABSciex) and bioinformatic analysis for metabolomic and proteomic studies.

**What are the proposed benefits and potential harms of this research and how does the benefits outweigh the harms?:**

Potential benefit: Generate an effective immune response to the patient's primary melanoma.

Risk-Breach of confidentiality**;** As with all research and sub-studies, there is a chance that confidentiality could be compromised; however, we are taking precautions to minimize this risk. All visits will be conducted in a private room to keep participant privacy.

Risk-Drug injections: Patients will be receiving an intralesional injection which may cause irritation and swelling at the site of injection. These discomforts are minimal and brief

Risk-Side effect of IL-2: Patients may feel mild flu like symptoms after receiving treatment, including fever, fatigue, headaches or muscle cramps. These symptoms are brief and general resolve within 48 hours

Risk-Drawing Blood; There is a possibility of pain, bruising, swelling or infection related to giving blood. These discomforts are minimal and brief.

Pregnancy/Fertility: FDA Pregnancy Category C. The effects of IL-2 on fertility and pregnancy is unknown and therefore pregnant women will be excluded from the trial and men and woman will be asked to use contraceptive protection during the two-week injection period.

Breastfeeding: it is not known whether IL-2 is excreted in human milk, therefore breastfeeding is contraindicated.

**Confidentiality:**

Non-identifying codes will be assigned to patient biological samples and personal health information (PHI) by the research offices at the QEII. The codes will not contain any identifiable information that can be traced back to the patient. The only file that links identifying codes with the patient will be kept on a password protected computer. A hard copy file will be stored separately in a secured location. Personnel who have access to identifiable patient information will be kept limited. Patient records and research results will be kept indefinitely.

**Compensation:**

Participants will be compensated $25 for each additional visit (2 visits) that is not part of their normal standard of care.

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