RESEARCH PROTOCOL

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

QEII Hospital
Nova Scotia Health Authority
Halifax
Canada

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Prepared by:

Gordon Simms,
Room 5E2, 5th Floor
Sir Charles Tupper Medical Building
5850 College Street
Halifax, Nova Scotia
B3H 4R2, Canada

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Lay Summary

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. Melanoma, the most aggressive form of skin cancer, has killed an estimated 1200 Canadians in 2016 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).

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. Systemically administered melanoma immunotherapies, such as IL-2, show promise but are plagued by morbid toxicity profiles and prohibitive pricing. Intralesional administration of IL-2 is a simple method to decrease the toxicity of IL-2 and improve efficacy over systemic administration, and may serve as a cost-effective treatment modality for pre-metastatic melanoma.

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 – and preventing metastases. *We believe that a "Proactive" pre-treatment strategy with IL-2 is the future of melanoma therapy.* 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 6,800 of which are Melanoma, the most deadly form of skin cancer.[2] It is estimated that in 2016, over 1200 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 academic dermatologists (Dr.s R. Langley, K. Purdy and P. Green) – 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 1200 expected deaths in Canada in 2016 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) Is pre-treatment of IL-2 on highly suspicious lesions effective in generating an adaptive immune response and preventing melanoma metastasis?

3) Sub-study – Is there a difference in specific immune modulators in patients receiving IL-2 pre-treatment compared to patients receiving a placebo?

Subject Selection:

Dr.s Green, Purdy and Langley (Dermatology) will identify and approach potential participants to recruit them to become part of the study. The participant population will include patients between 16 and 80 years of age with lesions highly suspected to be melanoma. Patients who are immunocompromised, have known autoimmune or inflammatory disease, or are undergoing immune-therapy for any other conditions will be strictly excluded from the study. We aim to include a minimum of 20 (up to 60) local participants over the next 12 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 IL 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 melanoma patients in response to intralesional IL-2, and whether that immunity can confer resistance to melanoma metastasis. This is a randomized, controlled, double blind trial where participants will be randomly assigned to a treatment group (IL-2: Proleukin (Aldesleukin)) or non-treatment group (saline placebo). Patients will be identified by Dermatology (Dr.s R. Langley, K. Purdy and P. Green) and interviews will be held at the surgery clinic (4th floor Dickson Center) QEII HSC, NSHA.

Randomization will be achieved through the pharmacy preparing treatment and placebo solutions. At the onset of the study, 20 opaque envelopes will be prepared by the investigators, randomized, and given to the pharmacy. Ten of these envelopes will contain instruction to prepare treatment (IL-2), and the other 10 will contain directions to prepare the injectable control (saline); in this way randomization of treatment and control groups will occur in the pharmacy. Once the randomization envelopes are depleted they will be replaced 10 at a time (5 treatment, 5 control)

until study conclusion. At the time of treatment randomization, the patient will be assigned a codified number (contained within the randomization envelope) to be used to track samples which will be collected and analyzed as a part of this study. This code will de-identify the patient and will allow samples to be analyzed without the risk of inappropriate exposure of participant's personal health data. When the study solution is prepared by the pharmacy, they will assign a codified ID which will be provided along with the syringe with the patient name labeled as study drug. The codified ID will be used to track samples that are analyzed at Dalhouise University, so patient identification information (such as name, address, or other identifying information) does not leave the hospital.

Treatments and incisional and fine needle biopsy will be conducted at the surgery clinic (4th floor Dickson Center) QEII HSC, NSHA.Treatment group patients will be treated intralesionally with IL-2 (Aldesleukin, Novartis Pharmaceuticals Canada Inc.) at a dose of 500,000 International Units (IU) in 0.1ml for 2 treatment cycles (1 treatment per week) prior to excisional biopsy; control group patients will be treated intralesionally with sterile saline (0.9% m/v) of the same volume for the same treatment cycles. Patient response to treatment will be monitored at each visit.

Prior to the first treatment and after excisional biopsy of the lesion, all patients will have blood (4 vials) and urine (25-50 ml) samples taken for proteomic and metabolomics analysis (**Figure 1**). Before delivery of the lesion to pathology, the lesion will be subjected to two fine needle aspiration biopsies: one directly into the lesion to be used for RNA analysis to assess the genetic profile of the suspected melanoma, and one in the clear margins of patient tissue obtained during the excisional biopsy. All samples obtained from pathology will be returned to pathology within 60 days of receipt, with the exception of the core needle biopsies which are destroyed in the process of analysis.

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. After histological diagnosis and staging, cells obtained as formaldehyde-fixed paraffin embedded sections (FFPE sections) from pathology will be obtained directly from pathology; these too will be labeled using the patients designated codifier.

FFPE sections from pathological analysis of the lesions will be analyzed using immunohistochemical staining techniques analysis to look for levels of tumor infiltrating leukocytes (TILs). Fine needle aspiration samples will be assessed for tumor genetic and epigeneitic profile. Blood and urine will be assessed for proteomic and metabolomic profiles, respectively.

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 and immunohistochemical 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. 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.

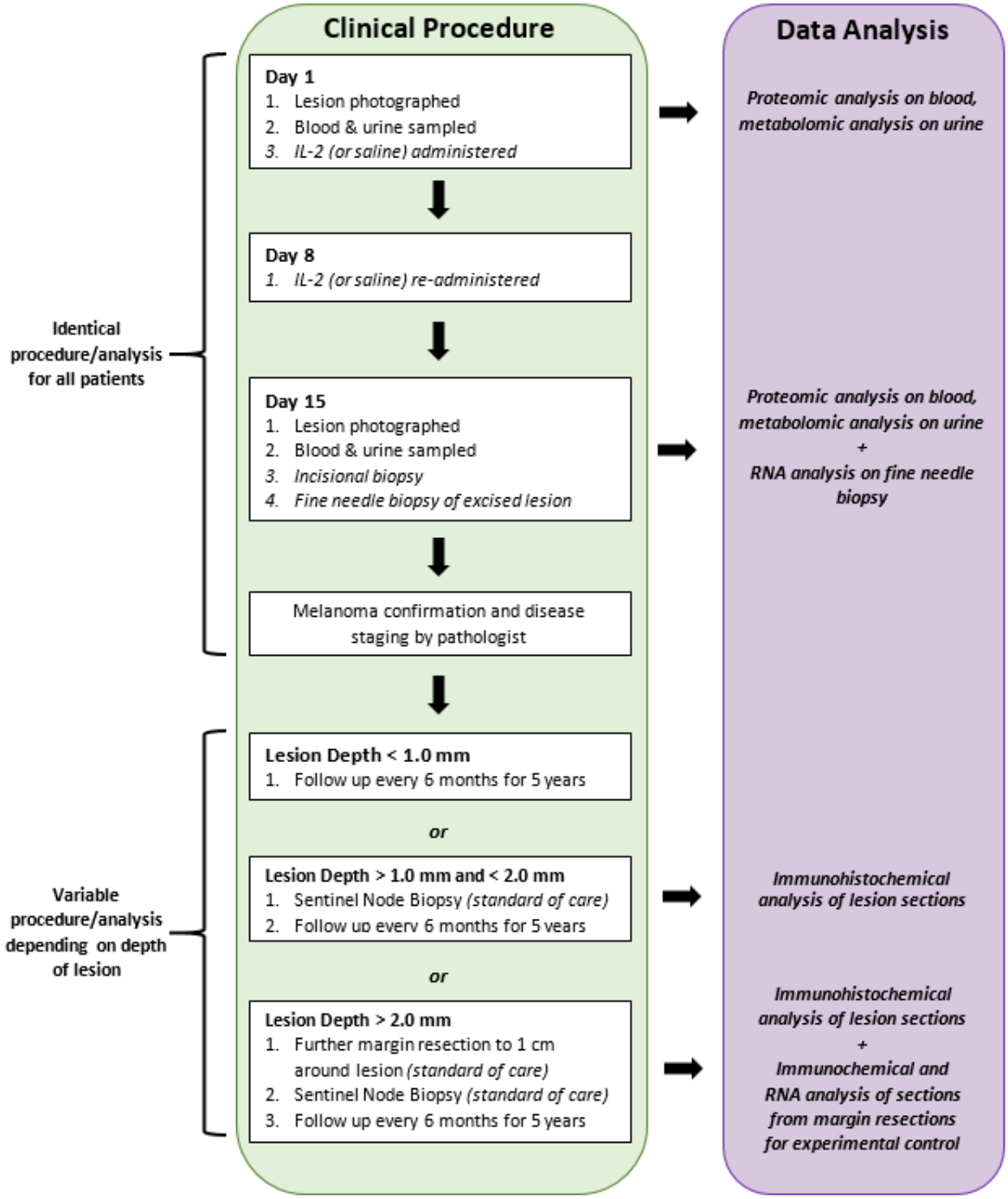

Figure 1 *– Clinical procedure and data collection/analysis algorithm for study participants.*

Analysis of Data

Outcome measure/study objectives:

Primary Outcome Measure:

1. Assessment of Lymphocyte Infiltration into Melanoma Lesions **[Time Frame: 1 year]** - The difference in lymphocyte infiltration between treatment and control groups will be compared. Lymphocytes to be assessed will include natural killer cells, T cells (CD4+ and CD8+), dendritic cells, macrophages (Mac-1+ and Mac-2+) and other immune mediators including PD-1, PD-L1 and FoxP3 expressing cells. TILs will be assessed using immunohistochemical staining of FFPE sections. Tissue controls will be obtained from the clear tissue margins obtained during the excisional biopsy. Immunohistochemical analysis will occur at Dalhousie's Histology and Services Research Lab (Sir Charles Tupper Medical Building, 11B). RNA analysis of the lesion will be compared to unaffected patient tissue obtained from the clear margins of the excisional biopsy to assess genetic changes resulting from the melanoma. RNA analysis will occur at Dr. Giancomantonio's laboratory (Sir Charles Tupper Medical Building, 11F11) at Dalhousie University.

Secondary Outcome Measures:

2. Assessment of Metastasis [Time Frame: 5 years] - All patients will receive biannual assessments for 5 years 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.

3. 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.

4. 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. Urine samples will be used in metabolomic studies to look for small molecule metabolites expression in patients before, and after treatment. Metabalome 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?:

Benefits: There is a chance that the patients placed in the IL-2 treatment group will benefit from the treatment, however there is a greater probability that the biological sample analysis will help future patients, if we can perfect and develop alternate preventative treatments.

Minimal Risk: Initial and follow up visits will be conducted in a private room to keep participant privacy.

Minimal Risk: Patients will be receiving an intralesional injection which may cause irritation and swelling at the site of injection.

Fertility: it is not known whether IL-2 can affect reproductive capacity

Pregnancy: FDA Pregnancy Category C. Studies in women and animals are not available for IL-2. Drugs should be given only if the potential benefit justifies the potential risk to the fetus. Women should be advised not to become pregnant while on therapy.

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:

There will be no compensation for patient participation in this study.

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